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FORAGE HANDLING, PRESERVATION AND STORAGE

PLANT CHEMISTRY

FEED UTILIZATION BY CATTLE

Preface

It is a pleasure to update our progress by bringing you these summaries of recent research. The U.S. Dairy Forage Research Center is a unique part of the national research program of the Agricultural Research Service, U.S. Department of Agriculture. The Center's mission is to build a knowledge and technology base for the dairy industry to fully exploit the use of forages in the production of milk. The Center has agricultural engineers, plant and soil scientists, microbiologists, ruminant nutritionists and a chemist working together to increase the efficiency of forage production and utilization by dairy farmers. We function in close cooperation with the Agricultural Experiment Stations of several states. The Center is located on the campus of the University of Wisconsin, Madison, and has "Cluster" locations in St. Paul, MN, Ames, IA, East Lansing, MI, and Ithaca, NY. The Center's research farm with facilities for 300 milking cows is located on 63 acres of USDA land on the banks of the Wisconsin River in Prairie du Sac, WI. An additional 1200 acres of adjacent land is utilized by the Center by agreement with the U.S. Department of the Army. The Center was established in 1980 and has made steady growth since. At present there are fifteen scientists: ten at Madison, and one at each of three Cluster locations, and two at the St. Paul, Minnesota Cluster location. Scientists hold faculty appointments in university departments and provide supervision for approximately 15-20 graduate students and 5-10 post doctoral fellows.

We are losing one of our colleagues this year, as Dr. Buxton in Ames, IA is taking a position on the National Program Staff of ARS in Beltsville, MD. Dwayne has been a very productive scientist and we will miss him very much. His research on questions of forage quality, forage production, and his more recent work with corn silage has always been very relevant to the needs of producers. We are uncertain about the future for the cluster position in Ames. From the U.S. Dairy Forage point of view, we would like to fill Dwayne's position with a new recruit. This, however, is being weighed against considerable pressure within ARS to consolidate outlying programs.

Another of our colleagues has also moved. Dr. Tilak Dhiman, having spent the last eleven years at the U.S. Dairy Forage Center, first as a post-doc and then as an assistant and associate scientist, has accepted an assistant professor position at Utah State University in Logan.

While we are saying farewell to two long time associates, we welcome Dr. Mark Powell. Mark is an agroecologist, and has been hired through a joint program supported by the University of Wisconsin College of Agricultural and Life Sciences and the U.S. Dairy Forage Research Center. Mark will be working with both ARS and college scientists to help develop and integrate research efforts in the area of nutrient management.

We received additional funding for our research efforts on questions relevant to low input, sustainable agriculture. Some of these funds will be provided to the University of Wisconsin via a specific cooperative agreement to help support an integrated cropping systems project. We will be developing research protocols in the next few months that address issues of sustainability, nutrient management, grazing, and cost of forage and milk production.

We are pleased and very proud of the way Center scientists from diverse disciplines interact and bring their collective insights to bear on the problems of forage production and utilization. This collection of research summaries illustrates the progress they are making in developing information to help dairy farmers utilize their resources more effectively. The research is intended to benefit producers of forage crops, dairy farmers and the consumers of dairy products.

Sincerely,

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Forage Production

Evidence of *Mycoleptodiscus* Root Rot of Forages in Wisconsin

C.R. Grau, D.K. Sharpee and R.R. Smith

Introduction

Soilborne plant pathogens are regarded as important causes of failures of newly established and mature stands of forage legumes in the North Central Region of the U.S. A *Mycoleptodiscus*-like fungus was recovered from decaying roots and stems of birdsfoot trefoil plants sampled from two-year-old yield trials in 1994 and establishment year stands in 1995 at the Arlington Agricultural Research Station, Arlington, WI. Although recognized in states south of Wisconsin, *Mycoleptodiscus terrestris* has not been implicated in poor health of forage legumes in Wisconsin. *M. terrestris* has been previously reported to be pathogenic on alfalfa, red clover and birdsfoot trefoil in Illinois (Gerdemann, 1954). The fungus has been reported to be pathogenic on birdsfoot trefoil in Missouri (Pettit, et al., 1969) and eastern U.S. (Carroll and Whittington,1991). Only the trefoil cultivar Dawn (Beuselinck, 1994) and the germplasm CAD (Beuselinck and Steiner, 1994) have been reported to have some degree of resistance (tolerance) to *M. terrestris.* However, no resistance has been identified in trefoil germplasm adapted to the northern area of the midwest. Forage legume germplasm has not been characterized extensively for reaction to *M. terrestris.* Our goal is to characterize the isolates for phenotypic and genotypic traits in relation to isolates from other geographic regions and host origins. This information will be used in a program to improve forage legumes for resistance to *M. terrestris*. Forage legume germplasm improved for resistance to *M. terrestris* can be used to assess the importance of this fungal pathogen in the upper Midwest.

Materials and Methods

Pathogen identification. A fungus resembling *Mycoleptodiscus terrestris* was recovered from root and stem tissue excised from birdsfoot trefoil plants. Excised tissue was placed on acidified potato dextrose agar (APDA) and incubated for 5-7 days. Isolates were grown on APDA, corn meal agar (CMA), microwaved soybean leaflets and irradiated carnation leaves for 7 days under fluorescent lights (12 hr light: 12 hr dark).

Isolates and germplasm evaluation. The virulence of eight isolates, four derived from field grown birdsfoot trefoil plants; one each from greenhouse grown red clover and alfalfa plants; and three from alfalfa-derived isolates provided by L.H. Rhodes, Ohio State University, Columbus, OH, was evaluated on eight forage legumes: alsike, berseem, kura, red and white clovers, alfalfa, birdsfoot trefoil and sweetclover. Three-week-old seedlings were inoculated with a mycelium/sclerotium suspension (one 100 mm standard plate/ l water) as a drench at a rate of 20 ml per 10 seedlings and incubated for three weeks at 25°C. Six-week-old seedlings were evaluated for reaction to the respective isolates on a scale of 1 to 5; 1 = no symptoms and 5 = a dead plant.

Results and Conclusions

Pathogen identification. All isolates recovered from birdsfoot trefoil produced sclerotia and conidia that were morphologically similar to *Mycoleptodiscus terrestris* isolates recovered from alfalfa. All birdsfoot trefoil isolates and two of the three alfalfa-derived isolates produced

conidia on carnation leaves but did not sporulate on PDA. One isolate from birdsfoot trefoil was tested on CMA but did not sporulate. Light was required for sporulation. All isolates produced sclerotia on carnation leaves, PDA and CMA.

Isolate-species evaluation. All forage species were susceptible to the *M. terrestris* isolates used in this study (Table 1), but alsike and kura clovers and alfalfa were less susceptible to this set of isolates compared to the other forage legumes evaluated. Symptoms varied from black decay of root tissues to severe wilting of the stem tissue resulting from root decay or black water-soaked lesions at the base of stems which completely girdled the stem.

Future Research/Plans

1. Isolates of *Mycoleptodiscus* recovered from forage legumes in Wisconsin will be compared to isolates from other regions of the United States and other species of *Mycoleptodiscus*. Isolates will be compared for differences in phenotypic traits such as spore and sclerotium morphology and virulence to specific forage legumes and compared to genotypic traits related to molecular markers.
2. A standardized method will be developed to select and characterize forage legume germplasm for resistance to *M. terrestris*.
3. An attempt will be made to improve forage legume germplasm for resistance to *M. terrestris*. This germplasm will be used to determine the role of *M. terrestris* on forage legume performance in the upper Midwest.

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Table 1. Response of eight legume species to nine isolates of Mycoleptodiscus terrestris.

SPECIES*	ISOLATE									Btw. Isolates SPECIES within spp.	
	Tre 94.1	Tre 95.1	Tre 95.2	Tre 95.3	Red 94.1	Alf 94.1	Rodalf 1	Rodalf 2	Rodalf 3	MEAN	LSD(5%)
	Disease Severity Index**										
Alsike Clover	3.50	2.54	2.57	2.02	2.57	2.66	2.95	3.14	3.01	2.77	0.51
Alfalfa	2.86	2.92	2.97	2.28	2.40	2.57	3.11	2.92	2.65	2.74	0.44
Berseem Clover	4.04	3.19	3.38	2.50	3.43	3.37	3.50	3.81	3.61	3.42	0.42
Kura Clover	3.44	2.67	2.91	2.47	2.91	2.89	3.32	3.00	3.17	2.97	0.40
Red Clover	3.95	3.09	3.20	2.72	3.39	2.88	3.83	3.88	2.96	3.32	0.33
Sweetclover	3.08	3.75	3.40	2.62	3.79	3.50	3.50	3.68	3.21	3.40	0.67
Trefoil	3.73	3.37	3.10	2.55	3.14	2.96	3.63	3.62	3.42	3.27	0.65
White Clover	3.67	3.19	3.30	2.36	3.25	2.79	3.58	3.24	3.01	3.16	0.59
MEAN	3.53	3.09	3.10	2.43	3.11	2.95	3.41	3.41	3.13	3.13	
LSD (5%)	0.76	0.58	0.24	0.39	0.42	0.42	0.4	.45	0.48	0.22	0.20
CV (%)	15.30	13.30	4.00	3.30	10.80	10.10	9.80	9.30	11.00	11.20	

**Disease Severity Index: 1 = healthy plant, 5 = dead plant.

ISOLATES: Tre 94.1 = Original trefoil isolate from 1994 field nursery
Tre 95.1 = Isolate obtained from field in Sept 95
Tre 95.2 = Isolate obtained from field in Sept 95
Tre 95.3 = Isolate obtained from field in Sept 95
Red 94.1 = Isolate from red clover inoculated with Tre 94.1
Alf 94.1 = Isolated from alflafa inoculated with Tre 94.1
Rodalf 1 = Alfalfa isolate #1 from Rhodes
Rodalf 2 = Alfalfa isolate #2 from Rhodes
Rodalf 3 = Alfalfa isolate #3 from Rhodes

*Alsike cv. common, Alfalfa cv.Vernal, Berseem cv. Bigbee, Kura cv. Rhizo, Red cv. Marathon, Sweetclover cv. Denta, Trefoil cv. Norcen, White cv. Ladino.

A Two Stage Selection Procedure for Resistance to Sclerotinia in Red Clover

R.R. Smith

Introduction

Sclerotinia crown and stem rot, caused by *Sclerotinia trifoliorum* Eriks., is one of the most destructive diseases on forage legumes in the eastern and north central U.S. and in Europe. Development of resistant germplasm in red clover (*Trifolium pratense* L.) has been difficult and slow. Until recently, the primary source of inoculum used in laboratory or greenhouse screening procedures was mycelium. However, the natural inoculum in the field is the ascospore. Recently, we developed a procedure for producing and storing ascospores in the laboratory which has allowed us to use ascospores as the source of inoculum in greenhouse screening tests (Smith et al., 1993; Marum et al., 1994). Selection for resistance in red clover using ascospores as inoculum has been slow but effective. It has been most difficult to generate uniform inoculation conditions whether using mycelium or ascospores, resulting in susceptible plants escaping infection and contributing to susceptibility in the subsequent generations. To overcome some of this procedural variability, a single leaf inoculation procedure has been proposed (Mouset-Declas et al., 1994). This becomes a very labor intensive procedure when screening thousands of plants for resistance. Therefore, a more efficient procedure is needed and this paper describes a two stage process which employs both a mass and a leaflet inoculation phase in the procedure.

Procedures

The two stage procedure involves a mass inoculation of two-week-old seedlings with ascospores followed by a replicated single leaflet inoculation of surviving seedlings.

Inoculum preparation and application. An isolate of *Sclerotina trifoliorum*, M1-B, collected from the Agricultural Research Station at Marshfield, WI was used for all procedures. Inoculum concentration for both stages was 10,000 spores ml^{-1} in a solution containing 10 g glucose l^{-1} and 3 drops of Tween 80 100 ml^{-1}.

Stage one. Mass Inoculation: Red clover seeds are sown in either sectioned or open plastic trays containing standard sterilized greenhouse soil. The trays are inserted in non-draining plastic flats such that the trays could be watered from the bottom with Hoagland's solution. Two-week-old plants were spray-inoculated with 80 ml of ascospore inoculum per pan. Pans were covered with clear plastic tops and sealed to retain 100% relative humidity. Plants are incubated at 15°C at 100% relative humidity in 12 hr day-length for 14 da. Plants are evaluated on a Disease Severity Index (DSI): 1 = healthy plant with no damage to cotyledons, 2 = slight necrosis with slight necrosis to cotyledons, 3 = moderate necrosis with death of cotyledons, 4 = severe necrosis, and 5 = dead plant.

Stage two. Leaflet Inoculation: Twenty-eight days after the mass inoculation, one leaflet from one leaf of each surviving plant is placed on moist, sterile filter paper in each of three separate 15 cm petri dishes sectioned to contain 10 to 16 leaflets per dish. One drop of ascospore inoculum at previously described spore concentration is placed in the center of each leaflet. The petri dishes are covered and sealed with parafilm and the leaflets incubated as prescribed for the seedlings in the mass inoculation. Fourteen days after inoculation leaflets are scored on a scale of 1 to 5 (1 = no necrosis, green leaflet; 5 = leaflet completely infected, light to dark brown). Plants with leaflet scores of DSI 1 or 2 are subsequently intercrossed to produce the next generation.

Results

Slight progress from selection for resistance to *S. trifoliorum* was realized in the first cycle of selection using the mass inoculation procedure

and standard phenotypic selection (Table 1). However, the process did not prove to be effective in the two subsequent cycles (C2 and C3) of selection. At this point we introduced the two stage inoculation procedure. The effectiveness of this procedure is reported in Table 2. Population R-C4 derived from plants declared as resistant by the leaflet inoculation step has significantly greater number of resistant plants than present in the MR-C4, S-C4 and C4 Mass populations. The mean DSI of population R-C4 (3.55) is significantly less than that for the other three populations and from the previous cycle of selection (C3). The response over all four cycles of selection is depicted in Figure 1.

Conclusions

The two stage ascospore inoculation procedure for identifying red clover seedlings with the resistant reaction to *S. trifoliorum* is effective and efficient. The initial mass inoculation step allows a breeder the opportunity to evaluate thousands of plants in a short period of time and the second step of leaflet inoculation effectively eliminates seedlings that have escaped infection in the earlier inoculation. Substantial progress should be realized with minimal effort using this procedure.

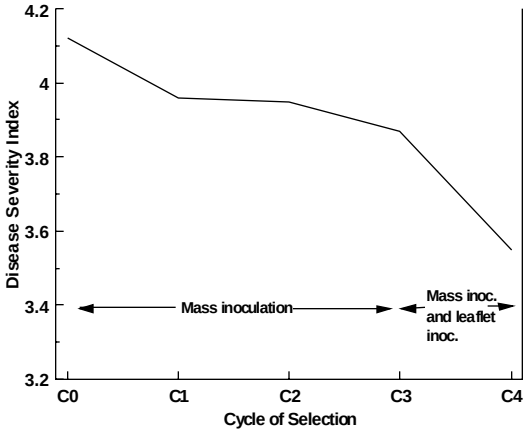

Figure 1. Response of four cycles of selection for resistance to Sclerotinia.

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Table 1. Response to three cycles of selection for resistance to S. *trifoliorum* in red clover using ascospores for inoculum.

	Percent plants with DSI of			
Popn.	1/2(R)	3(MR)	4/5(S)	Mean*
C0	10	10	80	4.16[b]
C1	12	12	76	3.96[a]
C2	14	13	73	3.95[a]
C3	12	18	70	3.87[a]
Arlington	8	22	70	4.14[b]

*Means followed by the same letter not significantly different at the 5% level.

Table 2. Response of populations derived from leaflet inoculation for resistance to S. *trifoliorum* in red clover.

	Percent plants with DSI of			
Popn.**	1/2(R)	3(MR)	4/5(S)	Mean*
R-C4	35	5	60	3.55[a]
MR-C4	12	10	78	4.30[c]
S-C4	8	3	89	4.55[d]
C4	24	6	70	4.00[b]
C3	19	5	76	4.11[b]
C0	10	5	85	4.41[d]

*Means followed by the same letter not significantly different at the 5% level.
**Popn. R, MR, and S are C4 popn. Derived from C3 plants determined to be resistant, moderately resistant, or susceptible by the leaflet inoculation test, respectively.

Bromide as a Tracer for Nitrate Uptake in Alfalfa

D.M. Magarian, J.F.S. Lamb, M.P. Russelle and J.M. Blumenthal

Introduction

New alfalfa germplasms that reduce nitrate (NO_3) losses to ground water and decrease fertilizer N requirements in crop rotations would be environmentally and economically beneficial to sustainable cropping systems. Although it has been demonstrated that alfalfa can absorb NO_3 from deep in the soil profile, there has been no economically feasible way to select alfalfa plants for differences in NO_3 uptake. Selection of alfalfa with high NO_3 absorption capacity under greenhouse conditions has failed to produce lines that differ under field conditions. The stable tracer, ^{15}N, is too expensive to use in the field, where thousands of individual plants must be measured individually.

Bromide (Br) has been used to trace nitrate movement in soil-water systems and is absorbed by many plant species. Our hypothesis was that Br could serve as an inexpensive tracer for NO_3-N uptake in N_2-fixing crops like alfalfa. We first developed a rapid means of extracting and analyzing Br from alfalfa tissue and then conducted three experiments in the greenhouse to test the hypothesis.

Methods

We tested several methods of Br extraction from alfalfa tissue, using flow injection analysis and a colorimetric detection technique for quantification. Results were compared to X-ray fluorescence spectroscopy analysis of finely ground tissue samples. We found that a 1 min. extraction in 25 mL deionized water with 0.25 g ground alfalfa tissue and 2 g activated charcoal produced Br analyses that correlated well with XRFS (r^2 = 0.97). This rapid extraction procedure removed only part of the total Br, so it is useful only in identifying differences among plants, not in determining total Br uptake.

Three experiments were conducted in the greenhouse with established alfalfa plants grown in soil/sand mixtures or sand only, and specific treatments within each experiment were repeated with other plants the following year. Experiment 1 was designed to test the hypothesis that the uptake of Br and N derived from NO_3 (using ^{15}N to monitor actual uptake of NO_3) would be related to the concentration supplied, and that the molar ratio of the tracer in the plant would reflect the ratio in solution. Four tracer concentrations at a constant molar ratio of 200 NO_3:Br were applied for one regrowth period. Experiment 2 was designed to test the hypothesis that Br uptake would be related to Br concentration in solution applied, regardless of a high concentration of NO_3 in solution. We applied four (yr 1) or 6 (yr 2) concentrations of Br with 5 m*M* NO_3 for one regrowth period. The third experiment was designed to test the hypothesis that plant-to-plant differences in NO_3 uptake are present in alfalfa populations. Solutions containing 5 m*M* NO_3 and 0.025 m*M* Br were applied for one regrowth period to 92 plants of Webfoot alfalfa in yr 1 and to 26, 29, and 34 plants of Webfoot, Agate, and Ineffective Agate alfalfa, respectively, in yr 2. Standard analysis of variance or regression analysis techniques were used to evaluate treatment effects.

Results and Discussion

Both Br and NO_3-N uptake increased during regrowth, as expected, due to dry mass increases in the shoot. Nitrate uptake responded in a sigmoidal fashion to increases in NO_3 concentration in the root zone, but Br uptake showed a linear response in the range of Br concentrations tested. These differences in uptake pattern and the disparity in physiological role of N and Br in the plant prevent the use of Br to trace NO_3-N uptake in crop management or physiology experiments.

The molar ratio of NO_3:Br in the shoot tissue closely reflected the changing ratio in nutrient

solution, regardless of the amount of time allowed for regrowth (Fig. 1). The one exception to this occurred when 5 micro*M* Br was supplied with 5 m*M* NO_3 (yr 2), and these data were not included in the regressions. Because these data apparently fit power functions, the intercept differences in Figure 1 indicate that germplasms responded differently to low ratios of NO_3:Br; the higher the apparent intercept in this log-log plot, the higher the initial slope of the response function. When three alfalfa germplasms were tested in yr 2, both of the N_2-fixing types, Webfoot and Agate, had similar intercepts, but these were lower than the non-N_2-fixing Ineffective Agate, indicating that Ineffective Agate accumulated more NO_3 than Br as the NO_3:Br ratio increased. Differences in apparent intercept among harvest dates in yr 1 suggest that relatively lower preference is shown for NO_3 uptake than Br as alfalfa regrows. In only one harvest (25 d of regrowth in yr 1) was the slope of the relationship different from the others.

Plant-to-plant variability for both NO_3-N and Br uptake was evident within each entry in both years. Plants that were high in NO_3-N uptake also had high Br uptake (Fig. 2). A hypothetical selection pressure of 5% for the population of 92 plants in yr 1 is shown in Fig. 2. These results show that individual plant selection for NO_3-N uptake using Br uptake as the selection criterion would result in minimal error.

Conclusion

Results of these experiments suggest that Br has promise as a tracer for NO_3-N uptake in an alfalfa plant breeding program. Selection should be made with plants of uniform maturity after several weeks of regrowth to allow differences to be expressed. In addition, a relatively constant supply of Br is necessary because Br uptake is directly related to Br concentration in the root zone. Our technique provides the first affordable means to select alfalfa for NO_3-N uptake in the field.

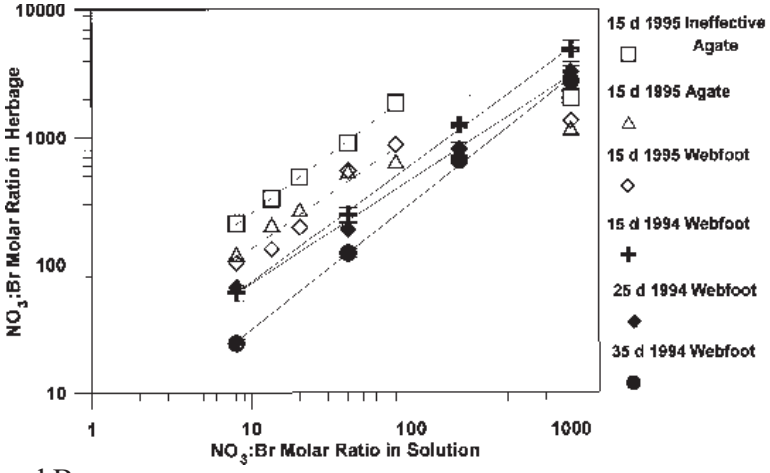


Figure 1. Molar ratio of NO_3-N:Br response to increasing Br concentrations and constant NO_3-N concentrations in solution treatments in two years, plotted on a log-log scale. Regression equations had the form $y = ax^b$, with $b = 0.94$ for all cases except 25 d regrowth in 1994, where $b = 0.82$ (in all cases $R^2 > 0.85$).

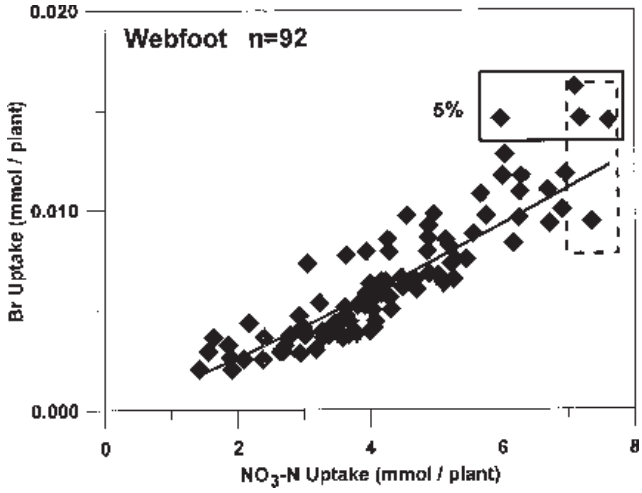


Figure 2. Scatter plot of NO_3-N uptake vs. Br uptake in yr 1. Selection for 5% of the population using Br uptake compared to NO_3-N uptake is shown in the outlined boxes. Regression equation was $y = 1.17(\log x)-6.77$, $r^2 = 0.79$.

Nutrient Management of Perennial Forages on Minnesota Dairy Farms

M.P. Russelle

Introduction

There is relatively little information available on a state-wide basis regarding fertility management of perennial forages by dairy farmers. Information available on manure and fertilizer use generally relates to corn acres. It is critical to know what fertility practices dairy farmers use on their perennial forages and what influences and constrains them in choosing those practices.

Method

Minnesota dairy farmers were surveyed by mail during August and September 1996. The questionnaire was mailed to 1007 dairies selected randomly by the Minnesota Agricultural Statistics Service from farms that had reported milking 30 or more dairy cows. A total of 354 surveys were found suitable for statistical analysis, representing 3.6% of all farms with less than 200 milking cows and 11.5% of farms with 200 or more milking cows. Because sampling intensity varied between these two categories, distributions of specific farm characteristics do not accurately reflect overall conditions in Minnesota. I divided the state into three areas, based on climate and general soil characteristics (Fig. 1).

Distributions of many individual farm characteristics were skewed, so both the median and mean are presented. Stepwise logistic regression was used to discern relationships among variables.

Results

Milk cow numbers ranged from 20 to 400 (P_{50} = 51, x = 69) and farmers reported having from 0 to 300 (P_{50} = 24, x = 34) replacement heifers. Rolling herd average ranged from 4000 to 11,800 kg milk (P_{50} = 8200 kg, x = 8400 kg) and is more likely to be high in larger herds, on farms where feed is tested for quality frequently, and as the area of permanent grass forages increases. It tends to be lower on farms that spread manure on grass forages. This latter characteristic may be related to other limitations on management (time, land area in corn, etc.), or it may be some direct effect, such as reduced feed intake due to palatability problems.

The percentage of forage land fertilized is greater in the cold Alfisol region, and when soil tests are used for information regarding fertilizer recommendations. In contrast, less forage land is fertilized where manure is topdressed on established alfalfa fields and on farms with permanent grass-legume mixtures.

Reported rates of preplant P and K fertilizer range from 5 to 70 kg P/ha (P_{50} = 18 kg P/ha, x = 23 kg P/ha) and 25 to 225 kg K/ha (P_{50} = 112 kg K/ha, x = 116 kg K/ha). More K is incorporated before seeding when farmers get information from fertilizer dealers and from the Extension Service and as the proportion of fertilized forage

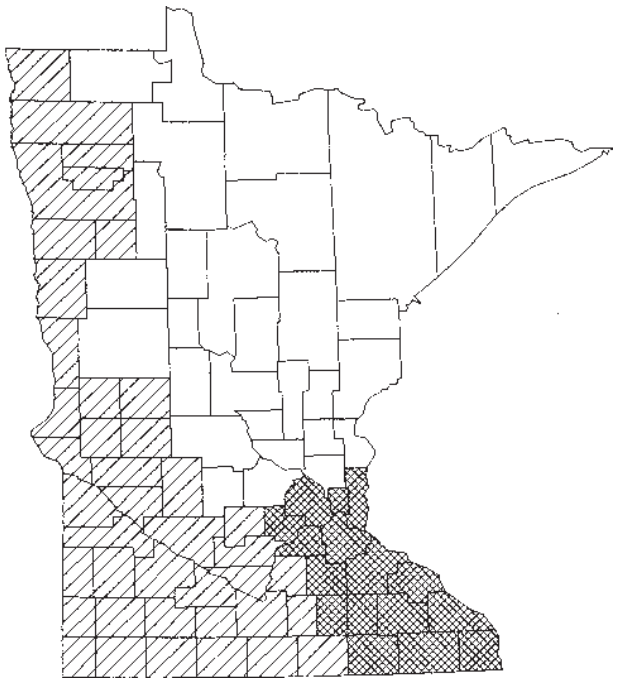


Figure 1. Three generalized soil areas of Minnesota: the cold Alfisol region (no shading); the warm Alfisol region (darkest shading); and the Mollisol region (medium shading).

land increases. The rate is smaller in the Mollisol region, which has high native soil K availability.

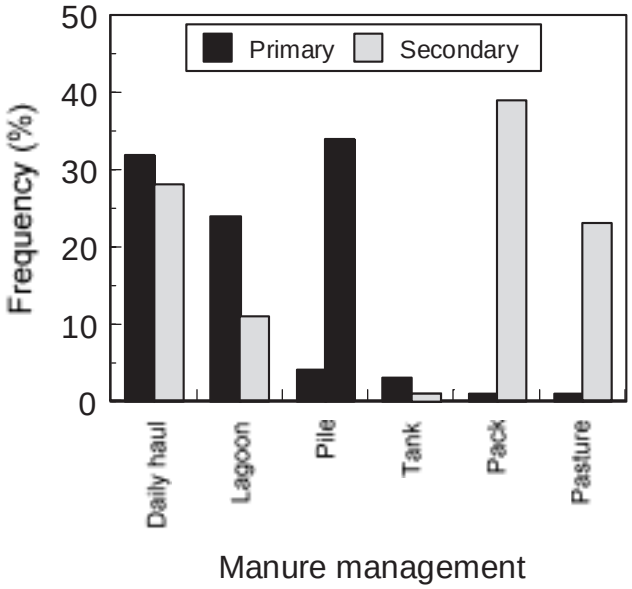
On established alfalfa, farmers reported using from about 5 to 70 kg N/ha (P_{50} = 20 kg N/ha, x = 24 kg N/ha), 5 to 80 kg P/ha (P_{50} = 21 kg P/ha, x = 27 kg P/ha), and 15 to 390 kg K/ha (P_{50} = 128 kg K/ha, x = 139 kg K/ha). Phosphorus rates are higher in the calcareous Mollisol region, on farms where a large percentage of forage land is fertilized, and when information on fertilizer rates is obtained from the Extension Service. Little variation in applied K could be explained by farm characteristics, presumably because nearly one half of all farms use K on established alfalfa. P and K fertilizer is applied almost entirely during the growing season and is spread all at once or in a simple split, mostly in September and October.

Nearly 80% of the reported soil tests exceeded 20 mg P/kg, at which point no additional nutrient is recommended by the University of Minnesota, and several exceeded 120 mg P/kg. Higher soil P levels were associated with manure storage in a pack and with higher percentages of fertilized forage land. Lower soil P is characteristic of farms in the Mollisol region and occurred on farms with daily or frequent haul manure systems. About one-half of the reported soil K tests were greater than 160 mg K/kg, the level at which no fertilizer is recommended. Soil K is related positively to pack and lagoon manure storage systems, with increasing proportion of forage land fertilized, and with increasing acreage of permanent grass forages.

The two manure handling systems ranked as most typical were daily or frequent hauling and lagoon storage of liquid manure (Fig. 2). Dairy farmers apparently rely more on daily or frequent manure hauling in the warm Alfisol area, as more forage land is fertilized, and as fertilizer dealers serve as more important sources of nutrient information. This also might be interpreted that farmers who rely on daily or frequent hauling are less likely to employ independent consultants than the personnel at the fertilizer distributor. Daily hauling is less likely as alfalfa land area increases.

Reliance on lagoon storage of liquid manure is more likely as herd size and rolling herd average increase, and is less likely in the warm Alfisol region and when information is obtained from farming magazine articles. Other manure handling systems rarely were listed as most typical, but were employed on many farms. Overall, manure storage facilities need to be emptied an average of twice a year, with a range of 1 to 26 times per year.

Minnesota dairy farmers are more likely to spread manure before seeding perennial forages when the typical manure source is manure pack in livestock facilities. Manure application both before seeding and as a topdressing on established forage stands is more likely with larger herds and on farms in the cold Alfisol area. Farmers are less likely to apply manure to perennial forages when a larger percentage of their forages received commercial fertilizer. Topdressing manure is less likely the more farmers rely on independent consultants for information on recommended fertilizer rates.



Figurs 2. Primary, or most typical (dark bars), and secondary (gray bars) types of manure management used on Minnesota dairy farms. Results of a survey done in 1996.

Topdressing manure on established grasses is less likely at higher rolling herd averages. About 10% of the respondents indicated that they topdressed at least a portion of their manure on forages during winter, but at least twice as many spread manure only during the growing season. The most frequently given reasons for topdressing manure on established perennial forages included the opportunity to spread manure in summer and to make good use of nutrients. In contrast, at least 20% of the respondents said that lack of time, lack of uniformity in spreading manure, increased weed problems, and lack of manure (due to use on other crops) were reasons for *not* topdressing manure on all perennial forage fields.

Although most dairy farmers reported using soil test results for information on fertilizer rates, the fertilizer dealers most frequently helped interpret that information. Farmers rely more on fertilizer dealers when they have more grass pasture, typically use a daily or frequent haul system for manure, and fertilize a higher proportion of their forage land. However, those who said pasturing their livestock is a typical way of managing manure are less likely to depend on employees of a fertilizer dealership for fertilizer recommendations. Similarly, farmers are more likely to use information from independent crop consultants when they fertilize a larger proportion of their forage land but also when they have larger herds. Those dairy farmers who spread manure on established forage stands are less likely to use an independent crop consultant for fertilizer recommendations. Extension, farm magazines, and experience were listed infrequently as the most important sources of information on forage fertilization.

Conclusion

Better knowledge of on-farm forage fertilization practices is needed to direct our future research and education efforts. As with all research, results of this survey should be verified by follow-up work in Minnesota and other states, and other areas where temperate forages are grown. This survey confirms earlier reports that many dairy farms have high soil test levels for P and K. Very high soil P concentrations may be reason to limit application of manure on these fields in order to preserve or improve surface water quality. Improved awareness of nutrient needs should increase the yield, persistence, and quality of perennial forages and improve the viability of livestock farming. Because fertilizer dealers are the most important source of nutrient recommendations to dairy producers in Minnesota, providing them with the latest information on nutrient management likely will result in the most rapid transfer of this information to farmers.

Nitrate Uptake Kinetics of Ineffectively and Effectively Nodulated Alfalfa

J.M. Blumenthal, M.P. Russelle and C. Bernasconi

Introduction

Alfalfa is one of the most important legume crops in temperate climates. It has high herbage yield and symbiotic N_2 fixation potential, is deeply rooted and provides high economic returns per unit land area. Efforts are under way to develop alfalfa for specific environmental quality goals, such as improved uptake of subsoil NO_3. Alfalfa forms a symbiosis with *Rhizobium* bacteria, which fix N_2 from the atmosphere, but alfalfa also absorbs inorganic N from the soil solution.

Because symbiotic N_2 fixation requires more energy than absorption and reduction of NO_3, the general understanding has been that legumes absorb inorganic N in preference to fixing N_2 gas. Research by our group has shown that alfalfa continues to fix atmospheric N_2, even with high rates of N fertilizer. Moreover, field experiments have demonstrated that ineffectively nodulated alfalfa, which cannot fix N_2 gas, absorbs NO_3 more efficiently than alfalfa with effective nodules. Greater NO_3 absorption occurred even when herbage yields of the ineffectively nodulated alfalfa were smaller than those of the effective types. The objective of this study was to determine whether NO_3 uptake kinetics could explain the disparity between these alfalfa types.

Materials and Methods

Plants of two effectively nodulated alfalfa germplasms, Saranac and Agate, and two ineffectively nodulated alfalfa germplasms, Ineffective Saranac and Ineffective Agate, were grown from seed for seven weeks in sand culture in the greenhouse, shoots were harvested about 5 cm above the crown, and plants selected for uniformity were transplanted individually into pots (PVC; 7.5 cm diam. by 30 cm long) filled with approx. 1200 mL nutrient solution containing 2 mg NO_3-N L^{-1}. The nutrient solution was aerated constantly and exchanged every 3 d. The plants were inoculated with *Rhizobium meliloti* strain 102F51. The plants were transferred to a growth chamber one week before NO_3 uptake kinetics were measured with day/night cycles of 14h/10h at constant 25° C and a relative humidity of ~ 90%. Photosynthetic photon flux during the day cycle was 550 µmol photons m^{-2} s^{-1} measured at the top of the canopy.

Two experiments were conducted. In the first, measurements were made on plants that received 2 mg NO_3-N L^{-1} every 3 d. Under such conditions the ineffective alfalfa germplasms were severely N deficient. To assay plants subjected to less N stress, a second experiment was conducted with plants receiving 2 mg NO_3-N L^{-1} daily for 2 wk before the assay period. Two hours after onset of the light period, the nutrient solution in the pots was exchanged with solution containing 0.6 mg NO_3-N L^{-1}, the weight of the pots was recorded, and starting 1 h later the solution was sampled (2 mL) every 10 min for the next 7 h, after which the weight of the pot was determined. Net decline in pot weight provided an estimate of H_2O transpiration. The following morning the nutrient solution was sampled again and the plant was harvested. Nitrate in the nutrient solution samples was measured spectrophotometrically, and total plant dry mass, Kjeldahl N concentration, and pot weight were determined. The time course of NO_3 disappearance from the nutrient solution was fit to a Lineweaver-Burke plot to determine the parameters of apparent Michaelis-Menten nitrate uptake kinetics.

Results and Discussion

In both experiment 1 and experiment 2 (Table 1), Saranac had the highest shoot yield and N content. Shoot yield and N content of Agate were intermediate. Shoot yield and N content of the two ineffectively nodulated, non-N_2-fixing germplasms were lowest and not different from one another. The ineffective germplasms were N

limited in both cases, resulting in 70% less shoot yield and 85% less shoot N content in experiment 1 and 67% less shoot yield and 82% less shoot N content in experiment 2, respectively, as compared to Saranac. However, the ineffective germplasms showed higher affinity for uptake of NO_3. In experiment 1, C_{min}, the minimal concentration of NO_3 below which there was no net uptake of NO_3, of the ineffective germplasms was one half that of Saranac and in experiment 2 C_{min} remained 22% lower than that of the N_2-fixing germplasms. The term I_{max} is the maximal uptake of NO_3 from nutrient solution by the plant when NO_3 concentration is nonlimiting (approx. concentration of NO_3 in the nutrient solution > 2 K_m). The ineffective germplasms had 126% higher I_{max} in experiment 1 and 171% higher I_{max} in experiment 2, respectively, than the effective germplasms. Additionally, we found differences in the water use efficiency between the effective and ineffective germplasms. The ineffective germplasms transpired 37% more water per unit shoot weight than the effective germplasms (2.60 vs. 1.95 g H_2O g shoot DM^{-1} h^{-1}).

Conclusion

Results of this study clarify the underlying physiological differences that lead to higher NO_3 uptake efficiency of ineffective alfalfa than N_2-fixing alfalfa we observed in the field. Ineffective alfalfa can deplete the nutrient solution to a lower NO_3 concentration and has a higher maximal NO_3 uptake capacity than effective alfalfa. Moreover, ineffective alfalfa transpired more water per unit shoot weight than effective alfalfa regardless of N nutrition. Higher water use in the field will cause more NO_3 to move to the roots, thereby increasing NO_3 availability for uptake.

Table 1. Shoot yield, shoot N content, and NO_3 uptake parameters of effectively and ineffectively nodulated alfalfa germplasms. Plants received 2 mg NO_3-N L^{-1} in nutrient solution every 3 d (Exp. 1) or every d (Exp. 2), respectively, before the uptake measurements. (n = 6).

Germplasm	Shoot dry weight g shoot^{-1}		Shoot N content mg N shoot^{-1}		C_{min} µ*M*		K_m µ*M*		I_{max} µg N min^{-1} plant^{-1}	
	Exp		Exp		Exp		Exp		Exp	
	1	2	1	2	1	2	1	2	1	2
Saranac	5.6	7.3	120	240	19	19	22	24	1.5	2.0
Agate	4.9	5.4	100	160	16	20	19	24	2.2	2.1
Ineffective Saranac	1.8	2.7	20	50	11	15	15	21	4.2	5.8
Ineffective Agate	1.6	2.1	20	40	9	16	13	21	4.2	5.2
$LSD_{0.05}$	0.7	1.4	30	30	4	2	3	2	1	2

Nitrate Losses Through Subsurface Tile Drainage in CRP, Alfalfa, and Row Crop Systems

G.W. Randall, D.R. Huggins, M.P. Russelle, D.J. Fuchs, W.W. Nelson and J.L. Anderson

Introduction

Drainage of excess water from the soil profile through subsurface tiles commonly is used to improve crop production on poorly-drained soils, particularly in the Upper Midwest. This drainage water can carry dissolved nutrients, suspended sediment, and pesticides from the field, and subsequently impact surface and ground water. Although nitrate-N (NO_3-N) losses have been monitored under fertilized crops in many areas, there is little information about the impact of perennial species, such as alfalfa and mixtures planted for the Conservation Reserve Program (CRP). Because perennial crops typically use more water than annual crops, and because neither CRP nor alfalfa receive fertilizer N inputs, we hypothesized that NO_3-N losses would be lower than when more typical annual row crop rotations were grown.

Methods

The experiment was conducted on a moderately well drained Normania clay loam (Aquic Haplustoll) at the University of Minnesota Southwest Experiment Farm at Lamberton, MN, from 1988-1993. Subsurface tiles had been installed in 1972 to allow monitoring of drainage in 15 individual 14- by 15-m plots, which were isolated to a depth of 1.8 m by 12-mil plastic sheeting. Five cropping treatments [continuous corn (C-C), corn after soybean (Sb-C), soybean after corn (C-Sb), alfalfa (Alf), and CRP] were established in the spring of 1988 in three replicates as a randomized, complete block design. Recommended hybrids, rates of seeding, fertilizer, and pesticides, tillage practices, and harvest schedules were used for grain (C-C, C-Sb, Sb-C) and hay (Alf) production.

No supplemental irrigation was provided. Tile flow rates and NO_3-N concentrations were measured and flow-weighted NO_3-N concentration was calculated. Total above ground dry matter and total N were measured, with estimates from 1-m^2 subplots in the otherwise undisturbed CRP plots. Standard analysis of variance was used to compare treatments.

Results

Growing season rainfall during the first two years was only 64 and 73% of the normal 530 mm, which limited crop yields, prevented tile drainage, and reduced stored soil water reserves. Tile flow did not resume until late May 1990. Above normal precipitation (by 13 to 66%) in the last three years resulted in higher yields (except in the cool 1993 season) and plentiful tile flow.

Above ground dry matter yields ranged up to nearly 15 Mg/ha in the corn plots (Fig. 1), of which about 58% was grain. Soybean yields ranged up to 10 Mg/ha, with an average of one third as bean dry matter. Alfalfa herbage yields peaked at 11.9 Mg/ha, whereas CRP standing above ground biomass attained only 5.3 Mg/ha. There was a close relationship between dry matter and N in the above ground crop (Fig. 1), but the slope for corn was only one third as steep as for alfalfa and soybean. These results support earlier conclusions that greatest potential N removal can be achieved with high yielding perennial forage species, like alfalfa. Both legumes likely fixed some atmospheric N_2, while absorbing some of the available soil N, but we cannot determine how much fixation or uptake occurred in this experiment. Alfalfa and CRP had about 65% less residual soil NO_3-N to a depth of 1.5 m in autumn than C-C, and corn/soybean rotations had about 17% less than C-C. In both cases, lower fertilizer N inputs may have contributed to lower residual soil N concentrations. Less water also was present in the soil profile in autumn under perennial crops than annual crops in all years but the wettest

(1993). Soil water reserves were reduced to depths of 3 m under alfalfa and 2.4 m under CRP.

As a result of increased water use and lower soil NO_3-N concentrations, NO_3-N loss in tile drainage was insignificant under both alfalfa and CRP, regardless of drainage flow (Fig. 2). In contrast, there was a close relationship between NO_3-N loss and water drained in the annual crops up to about 250 mm annual drainage. Total NO_3-N losses through tile drainage over the 6 yr period were 218 kg N/ha in C-C, 203 kg N/ha for corn/soybean rotations, 7 kg N/ha for alfalfa, and 4 kg N/ha for CRP. These losses represent about 25% of the fertilizer N rate applied to the annual row crop systems.

Flow-weighted NO_3-N concentration in tile drainage water during this study averaged 32 mg NO_3-N/L in C-C, 24 mg NO_3-N/L in the corn/soybean rotation, 3 mg NO_3-N/L in alfalfa, and 2 mg NO_3-N/L in CRP. In nearly all monthly time periods measured, the perennial crops maintained drainage water quality to concentrations below the public health limit of 10 mg NO_3-N/L.

Conclusions

In this experiment, we used the best practices available to optimize crop production and profitability, but NO_3-N concentrations in tile drainage water exceeded the public health limit in most instances under annual row cropping. Therefore, a production system of annual row crops on highly productive soils where biological influences can be significant will have difficulty attaining drainage water quality goals, even when prudent N fertilization is followed. Adding perennial species to the crop rotation will help reduce NO_3-N losses in tile drainage. In addition, perennial crop species will reduce the volume of water delivered to surface water through these drainage systems, thereby helping reduce flood potential in these areas.

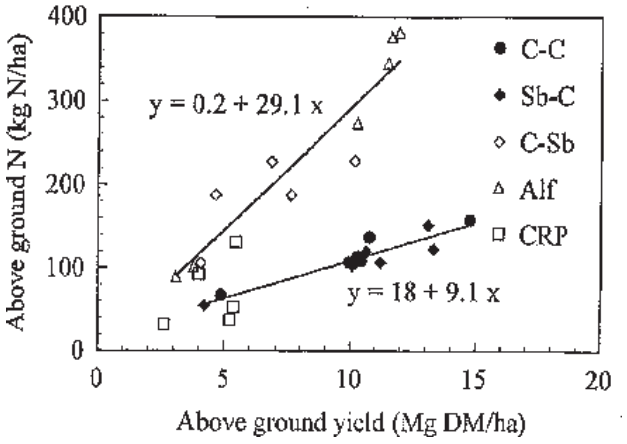


Figure 1. Relationships between above ground biomass yields and N content in five cropping treatments at Lamberton, MN, 1988-1993. In the corn/soybean rotation, data were obtained from the soybean phase in C-Sb, and from the corn phase in Sb-C. Regression lines were fit to alfalfa and soybean data or to corn data; CRP was omitted from both equations. Each regression had an $R^2 > 0.87$.

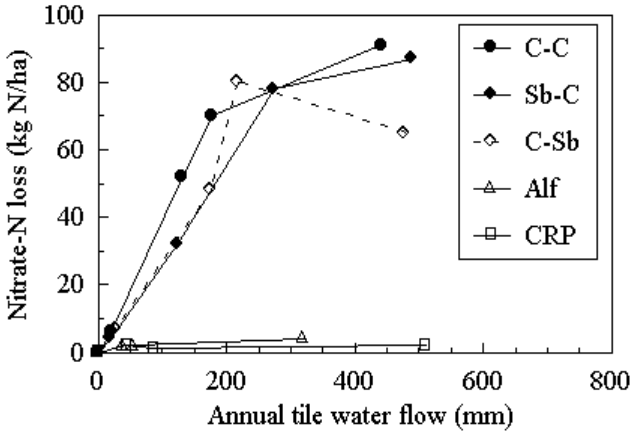


Figure 2. Relationship between annual flux of water and NO_3-N through subsurface drainage tiles in five cropping treatments at Lamberton, MN, 1988-1993. In the corn/soybean rotation, data were obtained from the soybean phase in C-Sb, and from the corn phase in Sb-C.

Forage Quality Characteristics of Inbred Corn Lines and Their Derived Hybrids

D.D. Redfearn, D.R. Buxton, A.R. Hallauer and J.R. George

Introduction

Corn is becoming more important as a forage crop in the United States. Up to 10% of the corn acreage is harvested as whole-plant silage. The decision regarding whether corn will be harvested for silage, however, is usually not made at the time of planting. This decision is often based on availability of forage from perennial forage crops such as alfalfa. This, in turn, depends on how well perennial forages survived the previous winter and how favorable growing conditions were during the current year. Corn stover (aboveground vegetation less ears) following grain harvest can also be an important feed resource for beef animals and sheep either as grazed forage or as conserved baled forage. Breeding programs emphasizing the development of corn hybrids specifically for forage have generally had low priority in the USA. Some companies have initiated breeding programs for improved corn forage in the USA and other companies evaluate forage quality of their hybrids and report this information to potential customers. Historically, those hybrids with high grain yields and high grain-to-stover ratios have been recommended as most suitable for forage. Nearly one-half of the aboveground dry matter of corn is stover. Thus, it should not be surprising that stover digestibility also greatly influences overall corn forage quality. Additionally, animals fed corn stover would benefit from plants developed for higher stover digestibility.

Information is needed on the relationship between forage quality characteristics of corn inbreds and their derived hybrids to increase the efficiency in developing corn hybrids for high forage quality. Decreasing fiber concentration or increasing fiber digestibility are the two most straightforward methods for increasing forage digestibility. This study was conducted to determine the relationship among forage quality of agronomically elite inbreds and their derived hybrids.

Materials and Methods

Twelve elite inbreds from the ARS/Iowa State University corn breeding program were used to develop twelve single-cross hybrids. These inbreds were B57, R227, NC258, Mo17, N28, B94, NC272, B52, B64, NC262, B79, and LAN496. Each inbred was used twice as a hybrid parent. Then, a replicated field study was conducted during 1994 and 1995 at Iowa State University Agronomy and Agricultural Engineering Research Center near Ames to evaluate both the inbreds and hybrids. Forage quality traits were measured by standard methods on both stover and whole-plant forage. Additionally, samples were fermented for 24 and 96 h to estimate the rapidly degraded neutral detergent fiber (NDF) and potentially degradable NDF fractions, respectively. The residual NDF ratio at 24 to 96 h was used as an estimate for rate of fiber digestion.

Results and Discussion

Genotypic variation for in vitro dry matter disappearance (IVDMD), NDF, and lignin/NDF were greater in stover and whole plant forage of the inbreds than in the hybrids. Additionally, whole-plant forage had larger genotypic ranges than stover. Generally, single-crosses of high forage quality inbreds resulted in high forage quality hybrids for whole plants (Fig. 1) with a poorer relationship in the stover (Fig. 2). There was a poor relationship between the estimate of rate of fiber digestion in the inbreds and hybrids. Significant genotype x year interactions occurred for several traits. NDF concentration explained less than 40% of the variation in stover IVDMD of both inbreds and hybrids. However, NDF concentration explained 62% of the variation for inbred whole-plant and 78% for hybrid whole-plant IVDMD.

Discussion

Given greater variation in forage quality among inbreds than hybrids and the positive relationship between whole-plant inbred and hybrid digestibilities, evaluating differences in agronomically elite inbreds should be an important criterion for selecting inbreds to develop hybrids with improved forage quality. Because of year x genotype variation, hybrids will need to be evaluated in several environments. The consistently negative relationship between fiber concentration and digestibility suggests that reduction of NDF may be an effective means of initially improving digestibility of forage corn. The lack of relationship between inbred rate of fiber digestion and hybrid rate of fiber digestion suggests that rate of digestion should be determined on hybrids. Additional research is needed to determine the stability of these relationships with other elite inbreds and environments. Because of the uncertainty of whether a hybrid will be used for forage at the time of planting, most forage hybrids will need to have high grain yield as well as high forage quality.

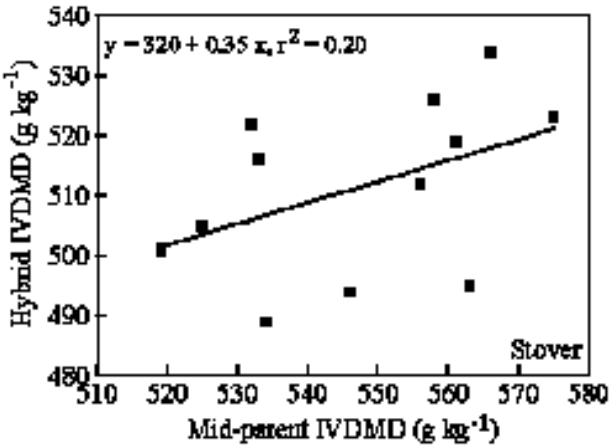


Figure 1. Relationship of stover digestibility of inbred parental average (mid parent) to that of the derived hybrid.

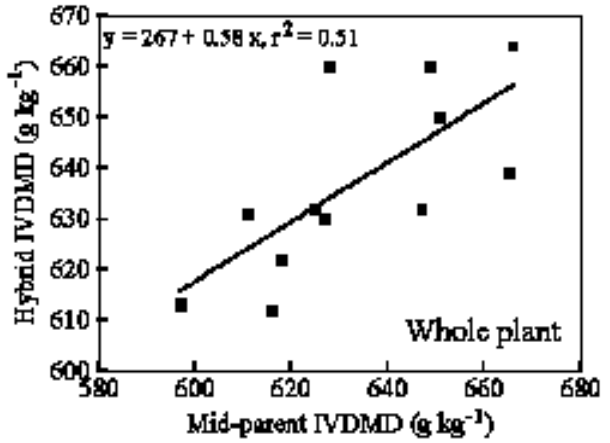


Figure 2. Relationship of whole plant digestibility of inbred parental average (mid parent) to that of the derived hybrid.

Economic Feasibility of Growing Forage Biomass Crops in Iowa

A. Hallam, I.C. Anderson and D.R. Buxton

Introduction

Today's primary energy resources of oil, coal, and natural gas are non-renewable. Besides being exhaustible, there is growing consensus that environmental concerns about global warming, air pollution, and acid rain are caused by combusting these fossil fuels. Much research is now focused on developing alternative energy resources that are renewable, dependable, safe, and environmentally sound. One such alternative is energy from biomass crops. Biomass can be used to generate electricity either by direct burning or co-firing with coal. It also can be fermented to a liquid fuel such as ethanol. Several forage crops are leading candidates as biomass crops. Development of such energy crops would result in alternative uses for land and forage crops. Despite its renewability and environmental sustainability compared with fossil fuels, biomass has not been widely accepted as a primary energy source because of its relative high price. One of the most expensive aspects of biomass energy is the cost of producing the biomass crop. This study evaluated the cost of producing several biomass crops in Iowa to identify those that can be grown at the lowest price per ton of biomass.

Materials and Methods

Enterprise budgets were calculated for several crops and cropping systems based on yields from experimental plots grown for five years at two sites in Iowa. The first site is in central Iowa on a low erosive, highly productive soil having a Land Use Capability Classification of I. The second is in southern Iowa on a lower productive soil with a Land Use Capability Classification of III. Machinery costs were based on average Iowa equipment use. Alfalfa was assumed to receive adequate phosphorus and potash during establishment for the life of the stand. Other crops received annual applications of fertilizer.

Results and Discussion

Results for four of the crops studied are presented in Tables 1 and 2. For this comparison, alfalfa was harvested three times per year, reed canarygrass twice, and switchgrass and forage sorghum were harvested once per year. Sorghum had the lowest break-even price in both central and southern Iowa followed closely by switchgrass. Land cost is an important factor in production cost estimation. It accounted for more than 30% of all production costs. Land costs were higher in central Iowa than in southern Iowa because of the higher capability of the soil. Because of the lower land price in southern Iowa, the break-even price was generally lower in southern Iowa. The soils in southern Iowa were sloping and would result in serious soil erosion when an annual crop such as sorghum is grown.

Conclusions

Forage sorghum is the most suited crop for biomass production in central Iowa. Even though it was the cheapest to produce in southern Iowa, potential for soil erosion on these sloping soils would limit production there. In southern Iowa, switchgrass may be the most suitable for growing low cost biomass and still protect the soil from erosion because of its perennial nature and sod forming root system.

Table 1. Estimated annual production cost ($/acre) in central Iowa.

Item	Alfalfa	Reed canarygrass	Switchgrass	Forage sorghum
Yield (t/ac)	4.85	3.67	4.97	7.01
Direct expenses				
Phosphorus		8.00	8.00	14.50
Potash		15.98	15.98	7.99
Nitrogen		15.00	15.00	15.00
Herbicide	3.62			15.76
Seed				2.45
Operator labor	14.97	11.45	6.07	13.18
Fuel	7.42	5.82	3.10	8.45
Implements	14.24	10.71	5.52	10.59
Tractors	8.97	7.08	3.77	10.19
Interest	1.14	2.58	2.36	2.60
Transportation	20.13	15.23	20.63	29.09
Total	70.50	91.85	80.43	129.81
Fixed expenses				
Implements	15.38	12.21	6.38	19.97
Tractors	15.92	12.57	6.69	18.09
Land	115.00	115.00	115.00	115.00
Total	146.30	139.78	128.07	153.06
Establishment cost (prorated)	65.89	15.92	5.63	
Total expenses	282.69	247.55	214.13	282.87
Break-even price ($/ton)	58.29	67.45	43.08	40.35

Table 2. Estimated annual production cost ($/acre) in southern Iowa.

Item	Alfalfa	Reed canarygrass	Switchgrass	Forage sorghum
Yield (t/ac)	3.99	4.34	4.61	7.41
Direct expense				
Phosphorus		8.00	8.00	14.50
Potash		15.98	15.98	7.99
Nitrogen		15.00	15.00	15.00
Herbicide	3.62			15.76
Seed				2.45
Operator labor	14.97	11.45	6.07	13.18
Fuel	7.42	5.82	3.10	8.45
Implements	14.24	10.71	5.52	10.59
Tractors	8.97	7.08	3.77	10.19
Interest	1.10	2.60	2.36	2.61
Transportation	16.56	18.01	19.13	30.75
Total	66.89	94.65	78.93	131.47
Fixed expenses				
Implements	15.38	12.21	6.38	19.97
Tractors	15.92	12.57	6.69	18.09
Land	80.00	80.00	80.00	80.00
Total	111.30	104.78	93.07	118.06
Establishment cost (prorated)	62.07	12.34	8.04	
Total expenses	240.26	211.77	180.04	249.53
Break-even price ($/ton)	60.22	48.79	39.05	33.67

Residual Cropping Effects on Corn Grain Yield

I.C. Anderson, D.R. Buxton, D.L. Karlen and C. Cambardella

Introduction

Cropping systems are known to have residual effects on soil quality and the productivity of following crops. Many of these effects have not been quantified for a range of cropping systems. This study was conducted to evaluate the effect of growing several annual and perennial crops on subsequent corn production.

Materials and Methods

Corn was grown on plots following 6 years during which one of 13 cropping systems was grown. The cropping systems were:

1. 'Arrow' alfalfa with subplots managed for either two or three cuts per year commencing the year following establishment.
2. 'Venture' reed canarygrass harvested twice per year, with subplots fertilized annually with 0, 70, 140, or 280 kg N ha^{-1} beginning the year after establishment.
3. 'Cave-in-Rock' switchgrass harvested once per year, with subplots fertilized annually with 0, 70, 140, or 280 kg N ha^{-1} beginning the year after establishment.
4. 'Sunny View' big bluestem harvested and fertilized as described for Cropping System 3.
5. 'M-81E' sweet sorghum harvested once per year, with subplots fertilized with 0, 70, 140, or 280 kg N ha^{-1}.
6. 'FFR 201' forage sorghum (sorghum x sudangrass) planted, harvested, and fertilized as described for Cropping System 5.
7. 'Aroostock' winter rye planted near mid October and harvested at anthesis during the succeeding mid May, followed by M-81E sweet sorghum planted in late May or early June. Subplots were fertilized with 0, 70, 140, or 280 kg N ha^{-1}. Half the N was applied in late March and the remainder before sorghum planting. The sweet sorghum was harvested in late September.
8. As described for Cropping System 7 but FFR 201 forage sorghum was planted instead of sweet sorghum.
9. 'Pioneer 3377' corn harvested for grain (with stover also removed). Subplots were fertilized with 0, 70, 140, or 280 kg N ha^{-1}. This cropping system was in a 3-yr rotation with soybean (Cropping System 10) and winter rye/sweet sorghum (Cropping System 11). Corn followed rye/sorghum in the rotation.
10. 'Hack' soybean grown in a 3-yr rotation with corn and winter rye/sweet sorghum. Soybean followed corn in the rotation and was harvested for grain with the stover also being removed.
11. Winter rye/sweet sorghum or winter rye/forage sorghum grown in a 3-yr rotation with soybean and corn. Subplots either contained or did not contain winter rye in combination with two N fertilization rates, 70 or 140 kg ha^{-1}. This cropping system followed soybean in the rotation.
12. M-81E sweet sorghum or FFR 201 forage sorghum intercropped into Arrow alfalfa beginning the year after alfalfa was established. Two subplots were planted to sweet sorghum and two to forage sorghum. Each sorghum was fertilized with 70 or 140 kg N ha^{-1}.
13. As described for Cropping System 12 but sweet and forage sorghum were interplanted into Venture reed canarygrass instead of alfalfa.

Cropping Systems 9, 10, and 11 were in rotation as described. All other cropping systems were on the same plots each year. In the fall of 1993, perennial grasses and alfalfa were sprayed with a herbicide and the entire site was moldboard plowed. In late April 1994, Pioneer 3377 corn was planted. After the plants were established, 224 kg N ha^{-1} from urea ammonium nitrate were applied to half of each plot.

Results and Discussion

Except corn following switchgrass, 224 kg current N ha^{-1} (N applied in 1994) resulted in near maximal grain yields regardless of the previous cropping system and previous N application rate. Highest corn yields occurred following alfalfa. There were marked carryover effects of previous N. Averaged for the eight cropping systems that received four rates of N yearly (Cropping Systems 2-9), corn grain averaged 7.6 t ha^{-1} when the previous cropping systems received no N and no current N was applied. When no current N was applied, corn grain yields increased 12, 29, and 41% when the previous cropping systems received 70, 140, and 280 kg N ha^{-1}, respectively. Application of 224 kg N ha^{-1} to plots that had not received N for 6 years increased corn yields by 71%. The carryover effect of previous N was particularly strong for corn following the three perennial grasses (Cropping Systems 2-4). Corn yields increased 44, 77, and 97% over the unfertilized checks when the three previous perennial grasses were fertilized with 70, 140, and 280 kg N ha^{-1} per year, respectively. This response is illustrated for reed canarygrass in Fig. 1. The four cropping systems that contained sorghum (5-8) had smaller carryover effects. Average corn yields were increased -2, 5, and 11% over the unfertilized checks when the previous crops were fertilized with 70, 140, and 280 kg N ha^{-1}, respectively. This type of response is illustrated in Fig. 2 for corn following forage sorghum. We were unable to document any consistent effects of the cropping systems on soil aggregate stability or nitrate N in the soil profile before the corn was planted.

Alfalfa has strong positive carryover effects on subsequent corn production. Much of this can be accounted for by the fixed N, but there appeared to be an effect beyond the N effect. The perennial grasses had marked N carryover effects. N was probably tied up by the root system of these crops and was released to the corn as the root systems decomposed. N carryover effects from sorghum were much smaller.

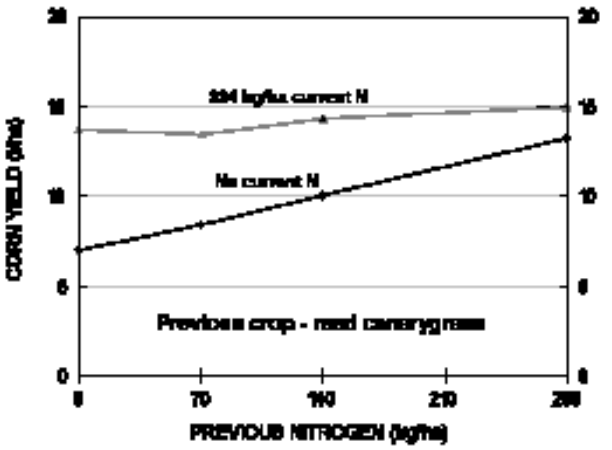


Figure 1. Corn grain yield following 6 years of reed canarygrass fertilized at four rates of nitrogen. The corn crop received 0 or 224 kg N ha^{-1}.

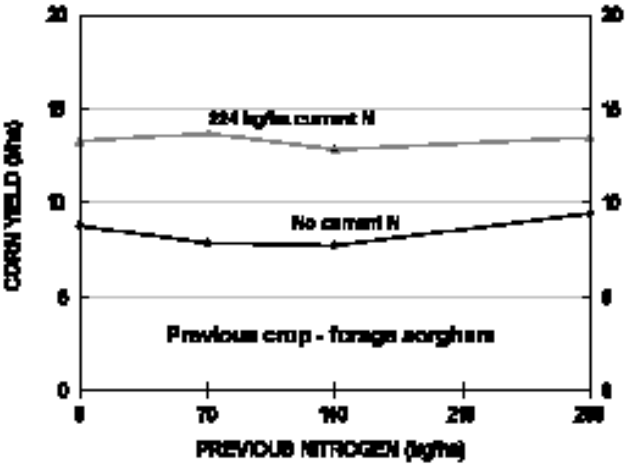


Figure 2. Corn grain yield following 6 years of forage sorghum fertilized at four rates of nitrogen. The corn crop received 0 or 224 kg N ha^{-1}.

Performance of Sweet and Forage Sorghum Grown in Monoculture, Double Cropped with Winter Rye, or in Rotation with Soybean and Corn

D.R. Buxton, I.C. Anderson and A. Hallam

Introduction

Potential for soil erosion restricts sorghum grown for forage or biomass to soils with little slope. Furthermore, because most of the aboveground crop is removed, soils may be at risk for water and wind erosion, and leaching of soil nitrate from the time sorghum is harvested until the next crop is planted and established; more than 6 months in the North Central Region of the United States. Double cropping with winter annuals after sorghum harvest can provide soil protection during the winter and following spring, tie up excess soil N, increase water infiltration, and may increase total biomass yield per year. Early spring growth of winter annuals, however, depletes soil moisture and may limit subsequent crop production. Winter rye is a potentially complementary double crop to sorghum. It is the most winter-hardy of the small grains and can provide high-quality forage. Winter rye can be seeded in fall after sorghum and become established before late-fall and winter temperatures prevent growth and development. It initiates new growth in late winter and early spring so that considerable biomass is produced before soil temperatures are warm enough to plant sorghum. This study was conducted to determine yield potential and composition of sweet and forage sorghum grown alone and when these sorghums are double cropped with winter rye and to determine the effect of growing sorghum in successive monoculture in relation to sorghum grown in a 3-year rotation with corn and soybean.

Materials and Methods

The experiment was conducted for five consecutive years on a Harps soil in central Iowa near Ames with a slope of less than 1% and on mixed Clarinda, Clearfield, and Grundy soils in southern Iowa near Chariton with 2 to 7% slope. 'M-81E' sweet sorghum and 'FFR 201' forage sorghum (sorghum x sudangrass) were grown in monoculture or double cropped with 'Aroostock' winter rye at four levels of N fertilization. Additionally, sweet sorghum, in a 3-year rotation with corn and soybean, was grown alone or double cropped with winter rye.

Results and Discussion

Sole forage and sweet sorghums were only moderately affected by N fertilization with average yields of 13.5, 16.1, 16.9, and 15.9 t ha^{-1} when fertilized with 0, 70, 140, and 280 kg N ha^{-1}, respectively. Conversely, rye/sorghum was extremely responsive to N, with highest yields at 280 kg N ha^{-1}. Winter rye yields averaged 3.0, 4.1, 4.6, and 5.0 t ha^{-1} and rye/sorghum yields were 72, 84, 95, and 110% of sole sorghum fertilized annually with 0, 70, 140, and 280 kg N ha^{-1}. Rye/sorghum yields were particularly sensitive to droughts. The low yield of rye/sweet sorghum relative to sole sweet sorghum during a drought year is illustrated in Fig. 1. Conversely, the high yield of rye/sweet sorghum during a wet year is shown in Fig. 2. Sweet sorghum grown in monoculture had yields similar to sweet sorghum grown in the 3-year rotation. Estimated annual soil erosion for sole sorghum determined by using the Universal Soil Loss Equation was 5 t ha^{-1} in central Iowa and 35 t ha^{-1} in southern Iowa. Planting rye before sorghum reduced the estimated loss to 3 t ha^{-1} in central Iowa and 22 t ha^{-1} in southern Iowa. Fiber concentration was higher in winter rye than in sorghum. Composition of sorghum was strongly influenced by environmental conditions of the study.

Conclusions

Winter rye can reduce potential soil erosion, but rye/sorghum yields are much more dependent on N fertilization and soil moisture than is sole sorghum. Though reduced, erosion was still too high to allow rye/sorghum production on the sloping soils of southern Iowa. Below normal

rainfall resulted in rye/winter yields that were consistently lower than those of sole sorghum. Including sweet sorghum in a 3-year rotation with corn and soybean had only limited impact on sole sweet sorghum production compared with sorghum grown repeatedly on the same plots for 5 years.

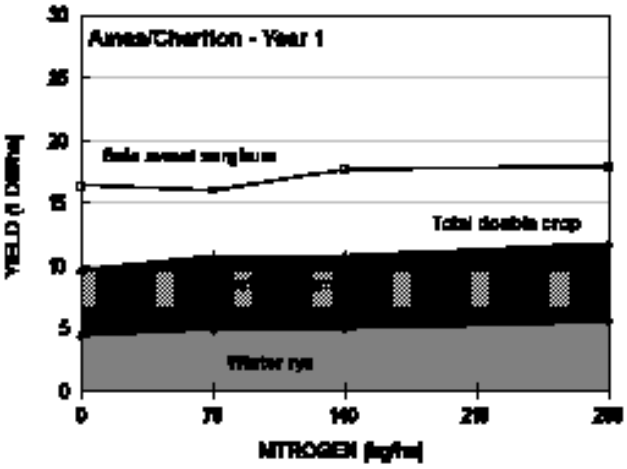


Figure 1. Yield of winter rye, winter rye plus double cropped sweet sorghum, and sole sweet sorghum at two locations in Iowa during a drought year.

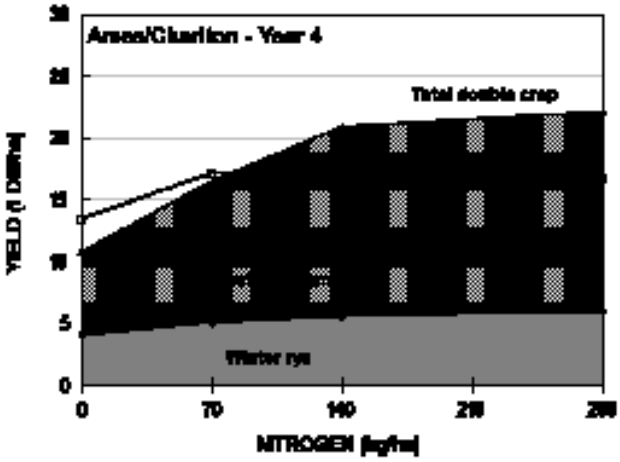


Figure 2. Yield of winter rye, winter rye plus double cropped sweet sorghum, and sole sweet sorghum at two locations in Iowa during a wet year.

Management of Mixed Species Pastures Under Rotational Grazing: Forage Availability, Nutritional Quality and Species Composition

V.R. Kanneganti, R.P. Walgenbach and L.J. Massingill

Introduction

Increasing numbers of livestock producers are considering grazing as a low-cost, profitable alternative to conventional dairying. This awareness has increased the demand for information on integrating pasture as a significant component of on-farm feed production. Mixed species pastures constitute a large and under-utilized resource on many livestock farms in the northeastern and north central U.S. These pastures are dominated by cool season species and are often termed as "natural" (i.e., not planted or tilled in a long time) or "permanent" pastures. Under good grazing management, these pastures have the potential to persist indefinitely while producing forage profitably.

In New Zealand and Europe, information on forage available for daily intake under grazing is provided to livestock producers for feed budgeting or enterprise planning. This information enables producers to match animal feed requirements with forage availability, which minimizes wasteful overfeeding of supplements while reducing feed costs and nutrient overloading on farms. Such information is scant for managing pastures profitably in the region.

The objective of this study was to provide livestock producers practicing intensive rotational grazing with quantitative estimates of forage available for daily intake by cattle grazing a mixed-species pasture.

Materials and Methods

Grazing management. A 50-acre pasture, located in Prairie du Sac, WI, was managed under a typical system of rotational grazing with lactating cows during May to October in 1994 and 1995. Rotation length was about 17 days each for grazing cycles 1 and 2, and about 30 days each for the other cycles. A new paddock was grazed each day. Forage allocation and animal movement were managed based on pasture height. Pasture height just before grazing averaged 7.1 inches while the stubble left behind after grazing measured 3.7 inches. The corresponding biomass amounts were 3373 and 2115 lb/acre. The pasture height was measured with a "rising" plate meter 12 in. x 12 in. which exerted a pressure of 0.51 lb per square foot on the canopy. To promote legume growth, fertilizer N was limited to 60 lb N/acre/yr, which was applied in two equal splits during the third and fifth grazing cycles.

Forage sampling. Forage availability, forage nutritional quality and species composition were measured across the grazing season on two 1-acre paddocks. Forage availability was calculated as: $G_i=(W_s-W_o)/T_r$, where, G_i is the forage availability during cycle, i (lb/acre/d), W_o (lb/acre) is the post-grazing biomass and W_s (lb/acre) is the pre-grazing biomass measured after a regrowth duration of T_r (d). Forage availability data presented in Fig. 1 and Table 1 represent daily amount of forage dry matter available for intake during a grazing cycle.

Nutritional quality of forage consumed was estimated as: $Q_{int} = (Q_s \times W_s - Q_e \times W_e)/(W_s-W_e)$, where Q_s and Q_e stand for crude protein (CP), neutral detergent fiber (NDF) or acid detergent fiber (ADF) as % dry matter in forage harvested before or after grazing, respectively. W_e (lb/acre) represents post-grazing biomass, and is equivalent to W_o for cycle, i+1. Q_{int} represents quality of forage consumed (CP, NDF or ADF, % dm).

Results and Discussion

Applied questions. What is the daily amount and quality of forage available for intake from a rotationally grazed mixed species pasture?

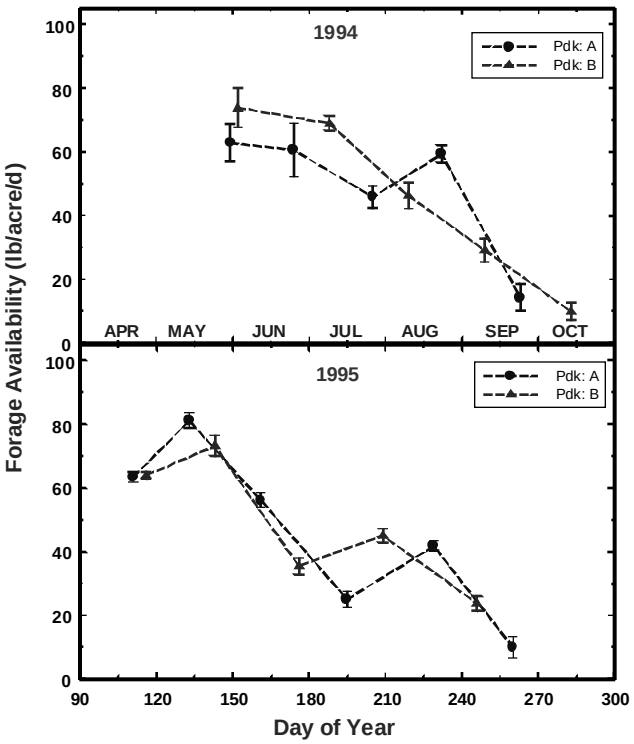


Figure 1. Forage available daily for animal intake under rotational grazing in 1994 and 1995. (Vertical bars represent standard error of mean.)

Quantitative estimates of forage available for daily animal intake measured across the grazing season are shown in Fig. 1 and Table 1. In most years, soil moisture may not be limiting pasture growth until late June, because soils are recharged with water from snow and early spring rains. Consequently, forage availability may be expected to be fairly uniform during this period. For the period of June-September, forage availability varies depending upon the duration and severity of moisture stress. Due to better distribution of rainfall during much of the grazing season in 1994, estimates of forage availability for 1994 may be representative of a "normal" year or a year with adequate rainfall while the data obtained in 1995 may reflect forage availability under extended periods of dry weather during summer. During September and October, temperature and light may be more limiting than soil moisture, and forage availability seems to drop steadily to about 10 lb/acre/d by the end of October.

Under adequate moisture conditions, these pastures have the potential to provide 3.8 t/acre of forage dry matter for intake under rotational grazing in a year (Table 1). Nutritional quality of forage consumed during the grazing season is shown in Table 2. Averaged over the years, forage consumed contained 22% CP, 45% NDF and 24% ADF.

What is the effect of intensive rotational grazing on species composition of the pasture?

Averaged over the two years, pasture dry matter was composed of 22% Kentucky bluegrass, 33% all other grasses combined (mostly smooth bromegrass and quackgrass), 13% legumes (mostly white clover), 27% dead matter and 5% all other species combined (Fig. 2). While more years of data are required to accurately assess the impact of rotational grazing on species composition, our data indicated little change in pasture composition during the grazing season, except under extended periods of drought. Under intense and long periods of dry weather which were observed in late August and September of 1995, dead matter fraction in the pasture increased to more than 50% while grass and legume fractions declined (Fig. 2). Bluegrass and white clover suffered the most under drought, but they recovered quickly to their original percentages upon return of favorable moisture conditions. Consequently, the pasture composition may remain fairly stable from year to year under the system of grazing management imposed even though significant but transient changes were observed within a grazing season.

However, under extended drought conditions, grazing low into the stubble may risk intake of poor quality forage because of the excessive amount of dead material in the forage.

Table 1. Daily and seasonal forage availability under rotational grazing in 1994 and 1995.

Grazing period	Grazing cycles[1] #	Forage availability 1994 Daily lb/ac/d	1994 Period lb/acre	1994 Total[2] lb/acre	1995 Daily lb/ac/d	1995 Period lb/acre	1995 Total lb/acre
05/01-06/01	2.0	68.3	1981	1981	70.5	2044	2044
06/01-08/15	2.5	59.0	4282	6263	40.7	2949	4993
08/15-09/15	1.0	34.3	1028	7291	25.2	755	5748
09/15-10/15	1.0	12.1	364	7655	nd[3]	nd	5748

[1]Number of grazing cycles during the specified grazing period.
[2]Total represents cumulative yield from start of the grazing season.
[3]No data; extended period of drought ended the grazing season.

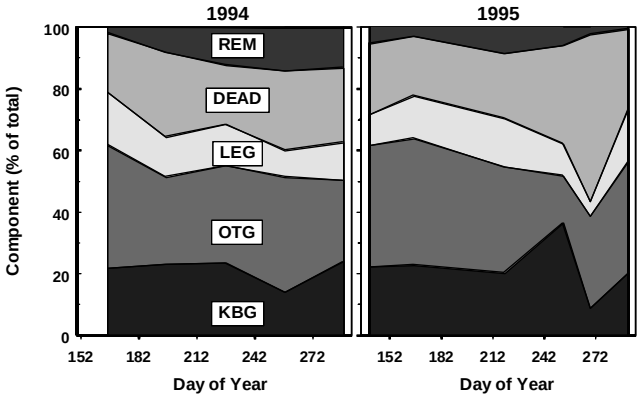


Figure 2. Changes in pasture species composition under rotational grazing in 1994 and 1995. [KBG = Kentucky Bluegrass; OTG = Other grasses (mostly smooth bromegrass and/or quackgrass); LEG=Legumes (mostly white clover); DEAD = dead matter; and REM = all other species (mostly dandelion or thistles)]

Table 2. Concentration of crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) in the forage consumed under rotational grazing in 1994 and 1995.

Sampling period[1]	CP	NDF	ADF
	- - - - % dry matter - - -		
		1994	
5/22 - 6/1	17.5	54.9	26.4
6/23 - 7/7	20.4	44.5	26.7
7/24 - 8/7	23.0	48.5	28.9
8/20 - 9/6	23.5	46.1	24.9
9/20	22.7	37.1	19.6
mean	21.3	47.2	25.9
SE	.94	2.06	1.40
		1995	
4/21 - 4/26	23.2	42.8	16.4
5/13 - 5/23	23.2	32.8	17.3
6/1 - 6/25	22.7	43.8	20.4
7/14 - 7/28	22.8	48.9	30.2
5/17 - 9/3	24.1	49.1	26.2
9/17	20.2	39.5	19.9
mean	23.0	43.1	21.9
SE	0.76	1.91	1.88

[1]Data from 2 paddocks averaged for grazing cycle.

Nitrogen Recovery by Orchardgrass From Dairy Manure

V.R. Kanneganti, S.D. Klausner and S.R. Kaffka

Introduction

With increasing manure production per unit cropland available for its disposal, greater recovery and recycling of manure N through crop uptake is needed on dairy farms to minimize environmental problems. On a 100-cow dairy farm, approximately 7 metric tons of N per year is not accounted for in mass balance equations. A substantial but unknown amount of this unaccounted N is being leached into the groundwater as nitrate.

Livestock producers need information on perennial grass forage crops which can utilize large quantities of manure N, yield good quality forage and fit into traditional crop rotations while allowing flexible and higher rates of manure application. Orchardgrass (*Dactylis glomerata* L.) is a well-adapted perennial crop with high yield potential. To develop effective manure management guidelines for this crop, its N recovery potential needs to be quantified. This study was initiated with the objective of quantifying N recovery potential of orchardgrass in response to liquid or solid dairy manure applied in spring with or without fertilizer N.

Materials and Methods

Dairy manure was surface applied at a rate to supply 150 kg total N/ha/yr to an established orchardgrass, either as liquid or as solid with bedding material, in April of each year in an experiment conducted for 2 years on a sandy loam soil in New Milford, Connecticut. Average composition of the manure is shown in Table 1. Manure treatments were superimposed with ammonium nitrate fertilizer applied at 0, 75, 150 and 300 kg N/ha in 1990, and 0, 150, 300 and 600 kg N/ha in 1991. The 3 x 4 factorial (no manure, liquid and solid manure, each superimposed with 4 rates of fertilizer N) was arranged in a randomized complete block design and replicated 3 times. Fertilizer P and K were applied according to soil test recommendation. Forage was cut in each plot from a 1m by 5m area to measure forage yield and N uptake (= N concentration in forage x forage yield). The crop was managed under a 4-cut system. N recovery by the crop was calculated by comparing N uptake on plots receiving manure or fertilizer with that of the check plot (no manure, no fertilizer), as follows:

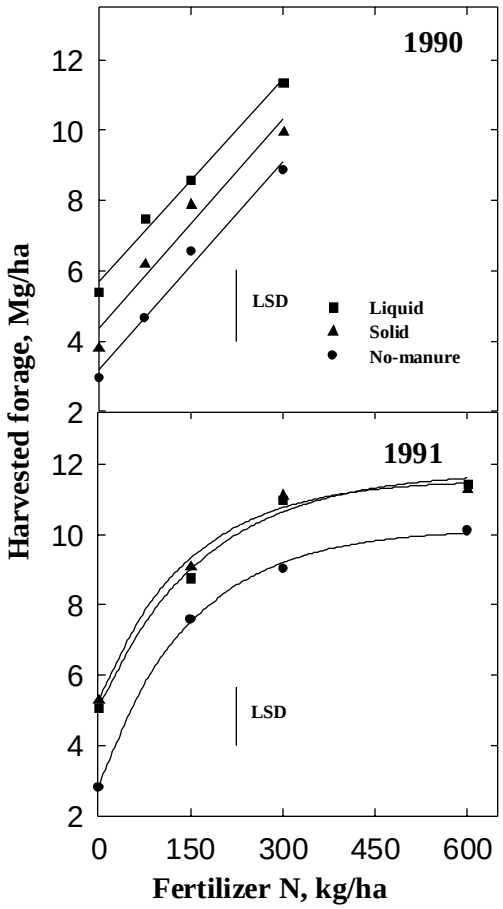
$$ANR = ((NTRT-NCHK)/NTOT)*100$$

where ANR is the apparent N recovery, NTRT is N uptake from manure or fertilizer treatment plot, NCHK is N uptake from check plot, and NTOT is the total N applied, all measured in kg N/ha/yr.

Results and Discussion

Forage yield. Effects of liquid and solid manure with or without fertilizer N on annual forage yield are shown in Fig. 1. In both years, yield increased significantly up to 300 kg fertilizer N/ha. In neither year was the interaction between N source and fertilizer rate significant, suggesting that the effects of solid or liquid manure on yield were similar at all rates of fertilizer N. Averaged over fertilizer N rates, liquid manure N increased dry matter yields over no-manure by 43% (2450 kg/ha) in 1990 and 23% (1684 kg/ha) in 1991 (Fig. 1). The corresponding values for solid manure N were 21% (1204 kg/ha) in 1990 and 24% (1812 kg/ha) in 1991.

N recovery. ANR represents the apparent amount of N recovered by the crop from fertilizer or manure. Averaged over the years, 40% of the 150 kg N/ha/yr of liquid manure N was recovered in forage in each year (Table 2). N recovery from liquid manure was 6% greater in 1990 than in 1991 (Table 2), suggesting that N losses were lower in 1990 than in 1991. In 1990, manure application was followed by a 33 mm rainfall over a 7 day period, which may have reduced volatilization losses of ammonia.



With solid manure N applied at 150 kg N/ha/yr without fertilizer N, 13 and 39% of manure N (19 and 59 kg N/ha, respectively) were recovered by the crop in 1990 and 1991, respectively (Table 2). In addition to N available from solid manure applied in 1991, more N was probably available from residues of the 1990 application, resulting in greater ANR in 1991. In contrast, no residual effects of liquid manure on forage yield or N uptake were observed (Fig. 1, Table 2).

Summary

Averaged over the years, orchardgrass recovered 40% of the liquid manure N and 26% of the solid manure N applied each year. On an annual basis, the crop recovered 430 kg soil N/ha from plots receiving liquid manure in combination with 600 kg fertilizer N/ha. This study demonstrates that intensively managed orchardgrass has the potential to absorb large quantities of manure N, suggesting that orchardgrass may be planted in fields that receive large quantities of manure on a regular basis.

Figure 1. Annual forage dry matter yield in response to fertilizer N with or without dairy manure. (Symbols represent average yields. Curves are best-fit functions (linear in 1990 and Mitscherlich in 1991):

1990:	*No-manure:*	$Y = 3172 + 19.7\ X;$	$R^2 = 0.90;\ RMSE = 777$
	Solid manure:	$Y = 4354 + 19.9\ X;$	$R^2 = 0.77;\ RMSE = 1230$
	Liquid manure:	$Y = 5685 + 19.3\ X;$	$R^2 = 0.75;\ RMSE = 1267$
1991:	*No-manure:*	$Y = 10175 - 7356\ e^{-.0068\ X}.$	$R^2 = 0.95;\ RMSE = 666$
	Solid manure:	$Y = 11573 - 6314\ e^{-.0069\ X}.$	$R^2 = 0.83;\ RMSE = 1129$
	Liquid manure:	$Y = 11812 - 6764\ e^{-.0059\ X}.$	$R^2 = 0.88;\ RMSE = 947$

where Y = forage yield, kg/ha; X = Fert N, kg N/ha; RMSE = Root of mean square error, kg/ha)

Table 1. Composition of liquid and solid dairy manures.

Manure	Dry matter content	Total-N	NH_4-N	Total-P	Total-K
	- - % - -	- - - - - - - - - % dry wt. Basis - - - - - - - - -			
			1990		
Liquid	11.2	5.75	2.20	0.76	2.87
Solid	28.1	1.99	0.57	0.78	2.31
			1991		
Liquid	6.9	4.30	1.45	0.97	4.40
Solid	13.9	4.00	0.74	0.84	3.86

Table 2. Apparent nitrogen recovery (ANR) by orchardgrass from fertilizer and from liquid and solid manures applied with or without fertilizer N.

	1990			1991			2 yr combined		
Manure type	Fert. N	Total[1] N	ANR	Fert. N	Total N	ANR	Fert. N	Total N	ANR
	- - kg N/ha - -		%	- - kg N/ha - -		%	- - kg N/ha - -		%
None	0	0	-	0	0	-	0	0	-
	75	75	55	150	150	95	225	225	82
	150	150	71	300	300	87	450	450	82
	300	300	64	600	600	59	900	900	60
Liquid	0	150	43	0	150	37	0	300	40
	75	225	59	150	300	66	225	525	63
	150	300	63	300	450	72	450	750	68
	300	450	62	600	750	49	900	1200	54
Solid	0	150	13	0	150	39	0	300	26
	75	225	40	150	300	65	225	525	54
	150	300	42	300	450	69	450	750	58
	300	450	52	600	750	49	900	1200	50

[1]Total N = fertilizer N + 150 kg manure N (for manure treatments), or
= fertilizer N only (for no-manure treatments)

Impact of High and Low Forage Diets on Dairy Farms

C.A. Rotz

Introduction

Dairy farms are complex systems with many components to manage. These components include crop production, harvest, storage, feeding, milk production and handling, manure management, tillage, and planting. Many of these components interact with weather and each other, so a change in one part of the farm may cause changes throughout other farm components. For example, a change in rations will affect the nutrient content of manure which can affect the fertilizer requirement and the productivity of future crops. In recent years with relatively low grain prices, dairy farmers have fed more grain than necessary with just enough forage to maintain proper rumen function. With the recent increase in grain prices, there is an opportunity to improve profitability by utilizing more forage and less grain in the dairy diet.

Materials and Methods

The long term performance and benefits of alternative dairy forage systems are best compared using DAFOSYM. DAFOSYM is a simulation model which integrates the many biological and mechanical processes on a dairy farm. Crop production, feed use, and the return of manure nutrients to the land are simulated over many years of weather. Simulated performance is used to predict the costs, income, and net returns or profit for typical dairy farms. By modeling several alternatives for the same base farm, those that provide maximum farm production or profit with good labor utilization and minimal effect on the environment are determined. All production and economic information is determined for each simulated year of weather. The distribution of annual values obtained can then be used to assess the risk involved in alternative technologies or strategies as weather conditions vary. The model was used to compare the whole-farm impacts of using high grain or high forage diets.

The modeled farm represented a typical 100-cow dairy farm in southern Michigan with 250 acres of owned land. The soil was a loam of medium depth. Essentially all forage and grain feeds required by the herd were produced on the farm using various portions of alfalfa and corn. Alfalfa was harvested using a four cutting harvest strategy with the first two cuttings harvested at a bud stage of development and the last two harvested in early bloom. Harvests began within 5 days of May 30, July 6, and August 20 for the first three cuttings and on October 15 for the fourth cutting. All cuttings were harvested as wilted silage.

Alfalfa was grown in a four year rotation with corn. Corn was harvested as silage and high moisture grain to fill the available silos, and additional corn was harvested as dried grain. Over the 25-year simulation, post harvest crop yields ranged from 4.0 to 5.7 ton DM/acre for alfalfa with a mean of 5.0 ton DM/acre. For corn silage, the range was 4.6 to 8.6 ton DM/acre with a mean of 6.1 ton DM/acre, and for corn grain the range was 75 to 155 bushel/acre for an average of 108 bushel/acre.

The herd included 100 Holstein animals (milking and dry) plus 85 replacement stock. Analyses were done with annual milk production set at 20,000 lb/cow. Feed rations were determined for two groups of heifers, a dry cow group, and three groups of lactating animals. A mobile mixing wagon was used to prepare total mixed rations (TMR) for each animal group. Cows were housed in a free stall barn and milked in a double six parlor. The culling rate of the herd was 35% which set the number of first-lactation animals at 35.

Simulations were conducted for two amounts of forage in lactating animal diets. First, a high forage diet was used where about 60% of the dry matter in lactating cow rations came from forage. Next, a high grain diet was assumed where forage was about 45% of the ration dry matter. The alfalfa area was reduced and the corn area was increased according to the feed needs. Simulations were done for 25 weather years using East Lansing, Michigan weather data. Prices were set to reflect long-term relative values of farm inputs and outputs in current dollars.

Results and Discussion

Simulated measures of farm performance included the feeds produced, those bought and sold to meet the needs of the herd, the milk production of the herd, and the manure produced and handled. The economic results included all major costs incurred and income from milk, excess feed, and animal sales. The net return to management was the difference between income and costs and thus provided an indication of farm profit.

With a shift from high grain to high forage diets, the need for forage increased. Alfalfa land was increased from 145 acres to 190 acres, and corn land was decreased from 105 acres to 60 acres. Alfalfa silage production increased from 600 ton DM to 800 ton DM while corn grain production dropped from 250 ton DM to 140 ton DM. With the use of more alfalfa, the soybean meal required to meet protein requirements dropped by about 10 ton DM per year. Due to the lower digestibility of high forage diets, manure production increased about 13%. With more alfalfa and less corn on the farm, the ratio of nitrogen available to that required to produce the corn increased from 2.7 to 5.6 while the farm maintained a good balance of phosphorus and potassium. This meant that much of the manure had to be applied to alfalfa to absorb the excess nitrogen.

Use of a high forage diet caused a slight decrease in purchased feed costs. This was due primarily to a need for less protein supplement when more alfalfa was fed. Because corn grain was custom harvested, the use of less grain reduced harvest costs. Fertilizer, chemical and drying costs also decreased with less corn on the farm. With all factors considered, the net return for the farm increased about $3,900 per year through the use of the high forage diet compared with the high grain scenario.

Conclusion

With rising grain prices, an obvious consideration is to use more forage and less grain in dairy rations. For farms where much of the grain is purchased from off-farm sources, there is a clear economic advantage for buying less corn. On farms where most of the feeds are produced on the farm, the advantage still exists, but it is not as great. When feeds are produced and used on the farm, the cost of production is much more important than the market price or value of those feeds. Use of more alfalfa forage can lead to considerable excess nitrogen on the farm which requires much of the manure to be applied to alfalfa to avoid loss of nitrogen to the environment.

Costs of Forage Production

C.A. Rotz and T.M. Harrigan

Introduction

Forage production costs form a major portion of the total cost of milk production on dairy farms. There are many methods available to produce forage, but most can be categorized as silage, dry hay, or grazing systems. For most dairies today, the primary forage is silage. Being a primary feed and a major cost, the economics of forage production can have a large influence on farm profit. Because of the many factors that influence production costs, a comprehensive analysis is needed to determine those costs. Such an analysis is best performed using DAFOSYM, a simulation model which integrates the many biological and mechanical processes on a dairy farm.

Materials and Methods

Long-term simulations with DAFOSYM were used to quantify the costs of forage production on Pennsylvania dairy farms. The model simulates crop production, feed use, and the return of manure nutrients to the land. Forage losses and nutritive changes, the timing of field operations, and the use of machinery, fuel, and labor are among the many factors tracked by the model to predict performance and resource use for typical dairy farms. Simulations were done for 22 weather years using Harrisburg, Pennsylvania weather data. Silage production costs were compared to those of alfalfa harvested in large round bales or grazed in a well managed rotational grazing system.

Two farms were modeled. The smaller farm included 100 cows plus 85 replacement heifers on 250 acres of owned land. Alfalfa was grown on 90 acres along with 160 acres of corn. The larger farm included 500 cows and 425 replacements on 450 acres of alfalfa and 750 acres of corn. Milk production was set at 22,000 lb/cow. Facilities included bunker silos for storing alfalfa and corn silages and a tower silo for high moisture corn. All alfalfa cuttings except the second cutting on the small farm were harvested as wilted silage. The second cutting on this farm was harvested as dry hay in large round bales. Corn was harvested as silage and high moisture grain to fill the available silos, and additional corn was harvested as dry grain.

A third farm was simulated which was a variation of the 100-cow farm that included grazing. All parameters of the farm were the same except that land use was changed to 150 acres of alfalfa and 100 acres of corn. Of the total alfalfa, 50 acres were grazed in the spring, 85 acres in the summer, and 150 acres in the fall after the third harvest. The remaining alfalfa was harvested with a three cutting harvest strategy using the same harvest methods and dates as used for the original 100-cow farm. Equipment costs included fence, watering equipment, and pasture clipping. Because field machinery was used less, cost was depreciated over 50% more time or 15 years.

Results and Discussion

Average costs of forage production on the three farms are illustrated in Figure 1. On the 100-cow farm, alfalfa silage was produced at an annual cost that varied over the 22-year simulation from \$76 to \$120/ton dry matter (DM) with a mean of \$85/ton DM. The predominant cost was that of machinery and the next largest cost was storage. Corn silage production was a little less costly, ranging from \$60 to \$124/ton DM with a mean of \$74/ton DM. Machinery, energy, and labor costs were less because mowing and raking operations were not required, and because the greater yield at harvest allowed equipment to be used more efficiently. Storage costs were a little lower for alfalfa because multiple cuttings allowed refilling of the silos, and thus more forage was stored in a structure.

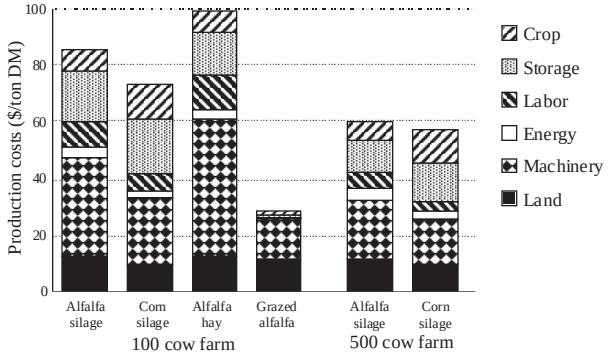


Figure 1. Production costs for different types of forage produced on two sizes of farms.

Hay production costs on the 100-cow farm were near $100/ton DM with a range in annual values from $83 to $167/ton DM. Machinery costs were higher because the baler and bale handling equipment were used relatively little. Hay can be produced more cost effectively when machinery is used for three or more cuttings per year.

Grazing was by far the lowest cost method of producing forage with a production cost near $30/ton DM. Annual values over the 22-year simulation ranged from $26 to $42/ton DM. Although grazing costs were quite low, they were only a portion of the total economic picture. Dairy farms in the northern U.S. cannot rely solely on grazed forage, so a substantial amount of hay or silage must be produced or purchased for the winter months. Despite the low production cost, grazing can reduce the profit of a high producing dairy farm by increasing other feed costs and reducing milk production.

On the 500-cow farm, where all four cuttings of alfalfa were harvested as silage using a self-propelled chopper and dump trucks for transport, alfalfa silage production costs dropped to $60/ton DM. The range in annual values was $55 to $82/ton DM. Machinery, labor, and storage costs were all reduced by producing forage more efficiently in greater amounts. Corn silage production costs were only a little less ranging from $48 to $95/ton DM with a mean of $57/ton DM. Again, compared to alfalfa silage, machinery, energy, and labor costs were less and storage and cropping costs were greater.

Conclusion

Silage production costs dropped as farm size increased with alfalfa silage costing a little more to produce than corn silage. The major cost in silage production was that of machinery which included depreciation, repairs and maintenance, and the next major cost was that of the storage structure.

Corn Silage Following First-Cut Alfalfa: A Forage Production Alternative?

J.C. Durling, Q.B. Hesterman and C.A. Rotz

Introduction

Corn silage is occasionally double cropped with first-cut alfalfa in the north central region of the US. With this system, corn is planted after first-cut alfalfa is harvested. This practice is of greater interest to growers with old or winter-injured alfalfa stands and limited alfalfa supplies. However, little information is available on forage yield and economic return of corn silage following first-cut alfalfa. This study was designed to: (1) evaluate forage yield and economic return of the corn silage following first-cut alfalfa system, (2) compare economic return from the alfalfa/corn silage double-crop system with returns from single-crop corn silage and four-cut alfalfa systems, and (3) determine the economic sensitivity of these systems to changes in soybean meal (SBM) and corn prices.

Materials and Methods

Field studies were conducted in Michigan in 1987 and 1988 to provide validation data for a longer-term computer simulation study. The experimental design was a randomized complete block with four replications. The forage production systems evaluated were: single-crop corn harvested as silage (Treatment C), alfalfa harvested four times at early to mid-bloom (Treatment A), and corn silage following first-cut alfalfa (Treatment AC).

Field studies were established in existing alfalfa fields following recommended production practices. Field operation, fertilizer, herbicide, and corn seed costs were summed for each system (Treatments C and AC). Land and alfalfa establishment costs were not included as variable costs since the crop was already available. Forage quality was determined with near-infrared reflectance spectroscopy. Market values ($/ton) of alfalfa and corn silage were determined as the cost of purchasing the same amount of crude protein (CP) and total digestible nutrients (TDN) in the form of SBM and corn grain. Prices of $240/ton for SBM and $2.53/bu for corn grain were used to reflect the long-term SBM dry matter (DM) to corn grain DM price ratio of 2:1. Gross margin ($/acre) for each treatment was calculated as market value times yield minus the selected variable costs of production.

Field data were used to validate the crop models using DAFOSYM (The Dairy Forage System Model) in predicting yields for this type of double crop application. DAFOSYM submodels were used to simulate crop growth using 1987 and 1988 East Lansing, Michigan weather data. Yields were simulated for Treatment C with the DAFOSYM corn growth submodel which used the CERES-Maize model, version 2.1. Yield and quality in Treatment A were simulated using the DAFOSYM alfalfa growth submodel which was based on the ALSIM 1, level 2 model from Cornell University. Alfalfa yield and quality and corn yield in Treatment AC were simulated using the respective alfalfa and corn models. Alfalfa growth was simulated as in Treatment A for the first cut. Available soil moisture predicted by the alfalfa model following the first cutting was carried into the corn submodel as the available soil moisture at corn planting. Market values and gross margin were determined as for the field study.

The DAFOSYM submodels were validated by comparing measured gross margins with predicted values. Studies in 1987 and 1988 provided six data pairs. A correlation (r) of .84 gave good support for the validity of the DAFOSYM submodels. Based on the accuracy of these predictions, a simulation was undertaken to evaluate these forage production systems over 26 years of East Lansing, Michigan weather.

Results and Discussion

Simulated forage yield and gross margin for the three forage production systems were determined

for the 26 weather years. Treatment C was the forage production system with the highest gross margin in 16 of the 26 years and with the highest average gross margin ($433/acre). Treatment A had the highest gross margin in 6 of the 26 years and Treatment AC had the highest in only 4 of the years. The 26-year average gross margin was $387/acre for Treatment A and $320/acre for Treatment AC.

Corn silage yield in Treatment AC averaged less than half of that in Treatment C. Economical yields from the corn silage following first cut alfalfa system were only attained during long, warm and wet summers. This type of weather pattern does not frequently occur in Michigan. The poor performance of corn in Treatment AC was consistent with other observations of the yield depressing effects of a forage legume preceding corn in a double-crop system. Reduced yields of corn following perennial legumes in most years is attributed to low early season precipitation and to the legume's use of soil moisture. High yielding legume crops grown in the spring decrease soil moisture content at corn planting time which often causes severe drought stress for the corn crop.

A sensitivity analysis was performed to determine how the forage system comparisons were affected by SBM and corn prices. Historic extreme SBM:corn price ratios of 3:1 and 1:1.5 were used. Within this range of price ratios, the number of years in which the doublecrop system had the greatest gross margin of the three systems ranged from 2 to 4 years out of 26. Therefore, the economic benefit of double cropping corn silage after first cutting alfalfa was not sensitive to feed prices, and this system was economically beneficial in very few weather years. Comparison of Treatment C and Treatment A was highly sensitive to SBM:corn price ratios. A low SBM:corn price ratio favored Treatment C in 22 of the 26 years, while a high SBM:corn price ratio favored Treatment A in 23 of the 26 years.

Conclusion

Producer interest in double cropping corn silage after first-cut alfalfa increases when forage supplies are limited and alfalfa stands have been injured by winter weather. The alfalfa/corn silage double-crop system was less profitable than single-crop corn silage and/or four-cut alfalfa in at least 22 of 26 weather years. Although the alfalfa/corn silage double-crop system has been successful in other areas, it cannot be generally recommended as an economic alternative for forage production in Michigan.

Comparison of Respiration Losses in Intensively Conditioned and Unconditioned Alfalfa

T.J. Kraus, R.E. Muck and R.G. Koegel

Introduction

Intensive conditioning of forage crops has several advantages: (1) accelerated drying rates, (2) increased fiber digestibility, (3) improved protein utilization, and (4) improved fermentation characteristics. Under good drying conditions, macerated alfalfa dried to 20% moisture (w.b.) in 6 h or less. Reaching good moisture contents (45 to 65%) for ensiling requires only 1 to 3 h of drying. With such short wilting periods, respiration losses are not a concern. However, when the wilting period is extended, due to unfavorable weather conditions or by mowing late in the evening, the forage will remain in a moist, aerobic environment for an extended period of time. Several studies indicate that a direct correlation exists between respiration rate and conditioning level of forage. Therefore, the question arises as to whether severely conditioning the forage leads to excessive respiration losses.

Under aerobic conditions, plant respiration causes the oxidation of hexose sugar to carbon dioxide and water. The stoichiometric relationship is: $C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2$ + 2870 kJ/Mole. Therefore, measurement of CO_2 output will enable the estimation of dry matter consumed during aerobic respiration.

Procedure

Figure 1 is a schematic of the apparatus used to measure the rate of CO_2 production from forage samples. The apparatus consisted of 6 components: a tank of compressed dry-air, an air flow regulator, a respiration chamber, a desiccant, an air mass-flow meter, and a mass spectrometer. Four respiration chambers were fabricated allowing multiple runs to be performed simultaneously.

Severely conditioned and unconditioned fresh alfalfa samples, approximately 1000 g each, were placed into the respiration chambers and allowed to respire for 48 h. Dry air was metered through the chambers to maintain an aerobic environment inside each chamber. The mass flow rate of the air and the concentration of CO_2 entering and exiting each chamber was measured at 15 min. intervals. This allowed the rate of CO_2 production to be determined.

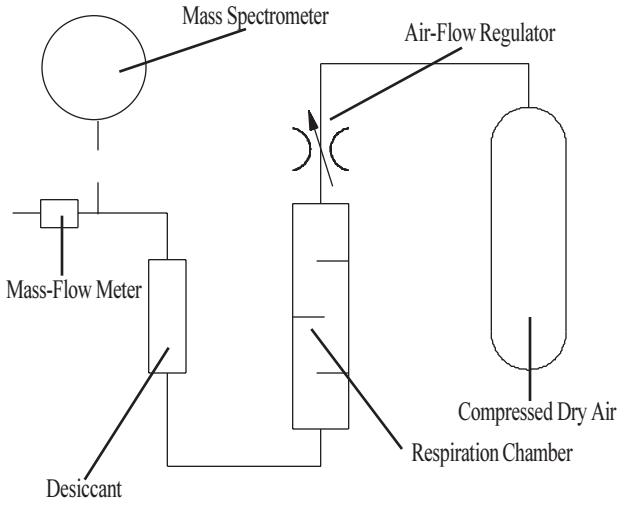


Figure 1. Respiration Apparatus.

Results

Production rates of CO_2 of severely conditioned and unconditioned fresh alfalfa at 31 °C are shown in Figure 2. The respiration of CO_2 from the unconditioned sample steadily decreased throughout the respiration period. However, the respiration of CO_2 from the severely conditioned material was different. During the first 8 h, the respiration rate of CO_2 of the severely

conditioned forage was lower than that of the unconditioned forage. However, after this initial lag phase, the production of CO_2 increased rapidly to approximately 2 times that of the maximum rate of the control.

Respiration losses of the stored carbohydrates and plant sugars occur for two reasons. First, undamaged cells continue to respire even though the upper portion of the plant has been severed from the roots. As the cells respire, they use carbohydrates as a source of energy. Second, microorganisms consume readily available sugars and carbohydrates that are in the soluble cell contents.

When forage is severely conditioned, many cell walls are broken, killing those cells. Therefore, one would expect initial plant respiration rates to be relatively low. It is believed the increased respiration rate of the intensely conditioned material after 8 h was due to accelerated microbial growth. The sudden decline in CO_2 production after 12 h and the rise after 30 h suggest shifts in dominant microbial populations as one group exhausts one substrate and then dies while another group rises using another substrate in the plant sap.

Conclusion

Initial respiration rates of severely conditioned forage were less than those of unconditioned forage. However, 8 to 10 h after conditioning, the rate of respiration of the severely treated forage increased rapidly to approximately 2 times that of the initial rate of unconditioned forage. This increase may have been due to accelerated microbial growth. If this rate of respiration continued over an extended period of time, there may be a significant increase in carbohydrate loss due to respiration in intensively conditioned forage.

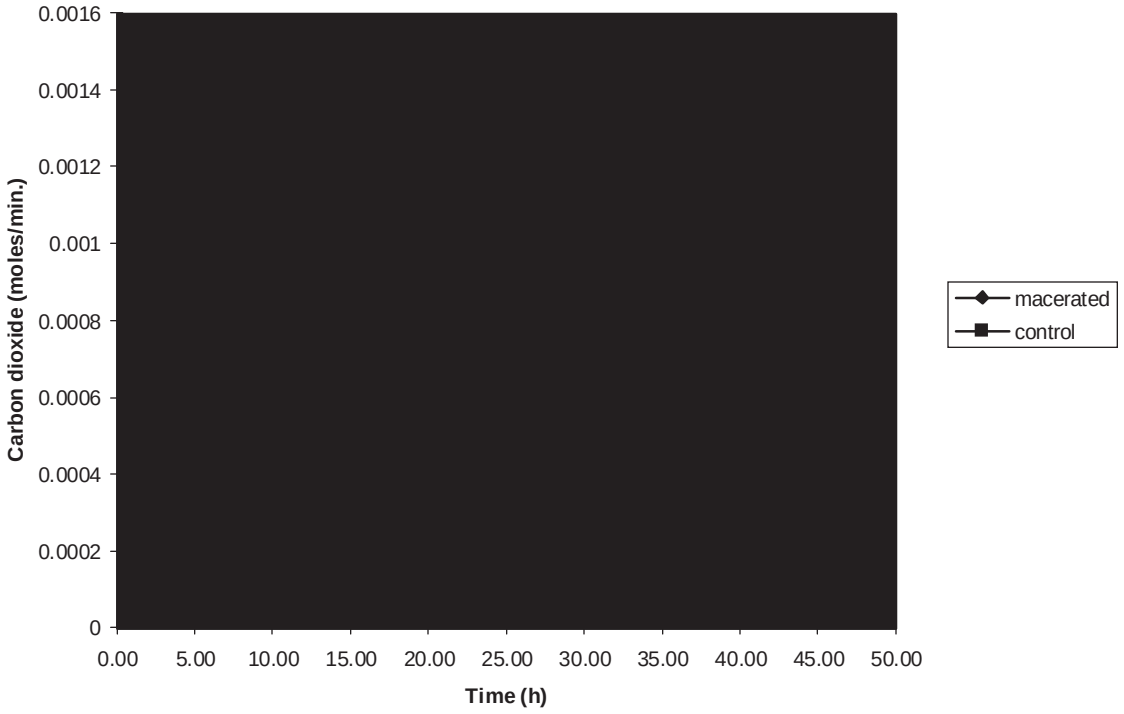


Figure 2. Carbon dioxide production from severely conditioned and unconditioned alfalfa at 31°C.

Maceration of Alfalfa Hay and Silage Improves Milk Production

D.R. Mertens and R.G. Koegel

Introduction

Previous experiments have shown that maceration of alfalfa improved digestibility in sheep, increased weight gain in late lactation dairy cows, and altered fermentation in the rumen. Maceration of forage increases the proportion of propionic acid produced during ruminal digestion which is similar to the result when additional grain is added to ruminant diets. This suggests that maceration of forage would provide the greatest benefit to animals with high energy demands such as cows in early lactation. The objective of this study was to obtain quantities of macerated and control alfalfa hay and silage that could be evaluated using high producing, peak lactation cows in a trial with adequate length and numbers of animals to detect differences due to maceration.

Methods

Third cutting alfalfa was harvested over a 28-day period to obtain 10 tons of dry matter as non rain-damaged hay and silage. Alternate rows were macerated or cut and conditioned, then harvested as hay or silage. Hay was harvested as small rectangular bales, and silage was stored in small plastic bags containing about 1000 kg wilted forage. To obtain consistent forage quality during the feeding trial, the least and most mature lots of forage were blended. Average forage composition was between 18 to 20% crude protein and 43 to 46% aNDF, and silages were 37 to 40% dry matter. Forages were mixed with high moisture corn, soybean meal, roasted soybeans (5% of ration dry matter) and minerals to obtain rations that contained 30% amylase-treated NDF and a minimum of 16% crude protein. Hays were chopped, and both hays and silages were fed as total mixed rations.

Forty-eight cows that averaged 80 days in lactation were divided into 12 groups based on parity and milk production within parity during a covariate period (covariate ration contained both hay and silage). One cow of each group was assigned to a ration containing control alfalfa hay, macerated alfalfa hay, control alfalfa silage, or macerated alfalfa silage. Cows were fed the rations for seven weeks.

Discussion

Statistical analysis of the data indicated that treatment differences were significant by the third week the cows were on their respective diets; thus, data for the last five weeks were averaged and used to determine the effects of maceration on animal response (Table 1). There were no differences in dry matter or NDF intake of cows fed macerated or control alfalfa forage. However, these early lactation cows responded to maceration of alfalfa with a significant increase in milk production. Although the cows fed macerated silage had lower milk fat percentages than control cows during the covariate period, there was a significant decrease in milk fat percentage for the macerated forage when least-squared means were compared. This has been a consistent response to maceration of alfalfa and may reflect the shift to lower acetate to propionate ratios in ruminal volatile fatty acids that was observed in previous experiments.

Although treatment differences were not significant, there was a trend toward greater positive body weight and condition score change for cows fed macerated forage compared to controls. This result agrees with previous studies. The combination of slightly lower dry matter intake with increased milk production and body weight change suggests that the energy utilization of macerated forages is greater than that of controls.

Conclusion

Maceration of alfalfa can result in greater milk production with slightly decreased milk fat percentages and a more positive energy balance. Not only does maceration appear to improve

energy utilization of alfalfa, but it also changes fermentation in the rumen in a way that results in greater propionate production similar to that which occurs when grains are fed. Thus, maceration may allow the substitution of some forage for grain in dairy rations.

Table 1. Least-squared means for animal responses during the last five weeks of the experiment when fed control and macerated alfalfa (averaged across hay and silage preservation methods (n = 24).

Variable	Control Treatment	Macerated Treatment
Chemical composition of total mixed rations (% of DM):		
Crude protein	16.9	17.7
amylase-treated NDF	30.8	29.7
Animal responses:		
Dry matter intake (kg/d)	23.4	23.2
aNDF intake (%BW/d)	1.18	1.19
Milk (kg/d)	34.5[a]	37.1[b]
4%FCM (kg/d)	32.6	33.5
Milk fat (%)	3.66[a]	3.37[b]
Milk protein (%)	3.12	3.05
Milk lactose (%)	4.85	4.83
Somatic cell count (1000)	161	181
Body weight (kg)	597	578
Body weight gain (kg/d)	-0.06	0.09
Condition score change (/mo)	0.12	0.15

[a,b]Values with different superscripts are different at $P < .05$.

The Potential for Ethanol Production From Alfalfa Fiber Derived From Wet Fractionation

R.G. Koegel and R.J. Straub

Introduction

Wet fractionation of forage crops allows biomass to be produced at very competitive prices due to the high values of the coproducts. The fractionation process consists of expressing juice from fresh herbage. The resulting fibrous fraction is high in cell wall constituents (cellulose, hemicellulose and lignin). It can be immediately field-dried and baled or pelleted if desired to minimize handling, transportation and storage costs. It is suitable for combustion, gasification, or enzymatic hydrolysis and fermentation to ethanol.

The juice fraction typically contains 25 to 30% of the dry matter in the original herbage depending on the severity of processing. It is high in protein and solubles and is almost fiber free. It can be used to produce both food-grade and feed-grade protein concentrates as well as other high-value products. The anticipated market value of the juice products, based on current prices of analogous products, greatly mitigates the production costs of the biomass fraction.

Wet fractionation of green herbage, the separation of fiber and juice, to produce protein concentrates has been researched and developed for more than a half century (Telek and Graham, 1983; Pirie, 1987). The technology and products are well known. For most of this period, however, emphasis was on making a crude, green protein concentrate either for livestock feeding or for supplementing the diets of humans suffering from protein deficiency. While the nutritional value of this product was undisputed, the economics of the process were, at best, marginal. Therefore, it was not, in general, commercialized.

Subsequent developments, however, appear to have greatly improved the potential for profitability:

1. Researchers have identified high-value juice products including soluble protein with good functional properties for human food (Knuckles and Kohler, 1982; Kohler et al., 1983), xanthophyll concentrates for use in the poultry industry (Crombie, 1995) and other products such as plant and animal growth stimulants, cosmetic substances, and pharmaceuticals (Koganov, 1992; Koganov et al., 1988).
2. Biotechnologists at the University of Wisconsin have demonstrated the possibility of adding genes to alfalfa which cause the transgenic alfalfa to produce industrially valuable substances, especially enzymes. Fields of alfalfa could thus become "bioreactors" or "enzyme factories" with the target enzymes recovered from the juice.

To date, transgenic alfalfa cultivars containing manganese dependent lignin peroxidase for biopulping, α-amylase for converting starch to sugar, cellulases for saccharification of ligno-cellulosics, and phytase to allow poultry and swine to utilize otherwise insoluble phosphorus in their grain-based rations, respectively, have been produced.

The use of forage crops, especially perennial legumes as a source of biomass, has a number of other advantages:

1. The need for nitrogen fertilizer, a high energy non-renewable input, is eliminated. Infrequent reestablishment of the crop minimizes the energy requirement for tillage and seed bed preparation.
2. Biomass can be field-dried and pelleted or cubed to minimize transportation, handling, and storage costs.
3. Forage varieties adapted to a wide range of environmental conditions exist, and production practices are established.
4. Machinery and methods for production and harvesting are available.

5. The excellent soil and water conservation characteristics of perennial forage crops are well recognized. This makes their production not only sustainable, but also desirable.

The economics of biomass production via wet fractionation is dependent on both unit prices and per hectare production of the various fractions.

Methods

In the spring of 1995 at Madison Wisconsin, four plots of approximately 42m^2 each were established in an alfalfa field which had been seeded the previous year. The first plot was mowed on May 22 and May 21 in 1995 and 1996, respectively, with successive plots mowed at 8-9 day intervals. This was done to "stage" the area as would be necessary in a large scale operation where it is required to keep harvesting and processing equipment working steadily at near capacity during the entire growing season. Each plot was then harvested at approximately 35-day intervals after its initial harvest for a total of four harvests per plot. The herbage from each plot was weighed and immediately macerated using a double rotor, rotary impact macerator (Koegel and Straub, 1994). Juice was then expressed by running the macerated herbage twice through a 15 cm diameter Rietz screw press. Herbage, juice, and fibrous fraction samples were oven-dried at 105 ^{o}C for 24 hours to determine dry matter content.

The following analyses were carried out on the fibrous fraction: (1) neutral detergent fiber (NDF), (2) acid detergent fiber (ADF), (3) acid detergent lignin (ADL), (4) nitrogen, and (5) ash.

The following definitions were used: (1) hemicellulose = NDF - ADF, (2) cellulose = ADF - ADL, (3) lignin = ADL, and (4) protein = 6.25 x nitrogen. Solubles were determined by difference.

Results

Per hectare dry matter yields of herbage and juice for 1995 are 13.5 t/ha and 4.2 t/ha, respectively, for an overall juice:herbage DM ratio of 0.31. In 1996, herbage dry matter yield was 12.9 t/ha and juice DM yield was not measured.

Table 1 gives the composition of the biomass or ligno-cellulosic fraction resulting from wet fractionation of the four harvests of each of the four plots in terms of cellulose, hemicellulose, lignin, protein, ash, and solubles (by difference). These are also converted to per hectare yields based on 9.45 t/ha of fibrous fraction.

Table 2 shows the potential per hectare production of ethanol based on yields of 85% of the stoichiometric. The soluble dry matter, which has not been characterized, was assumed to yield at the same rate as cellulose.

Wyman et al. (1993) give ethanol yields (l/t) of cellulosic biomass as 338 for "reference case" and 497 for "improved technology." Multiplying these yields times the 9.45 t/ha of alfalfa fibrous fraction gives 3194 l/ha and 4697 l/ha, respectively, which bracket the 4200 l/ha shown in Table 2.

Wyman et al. (1993) give approximate compositions of three classes of lignocellulosics: "agricultural residue," "hardwoods" and "herbaceous plants." The ratios of hemicellulose to cellulose for these three groups are approximately 1:1.2, 1:2.2, and 1:1.5, respectively, and the sums of hemicellulose plus cellulose are 70%, 73% and 75% of the total weight, respectively. In the case of the fibrous fraction of alfalfa, the ratio of hemicellulose to cellulose is 1:1.9 and the sum of hemicellulose, cellulose, and solubles is approximately 72% of the total weight. The lignin concentration of the alfalfa, at 7.8% is half or less than that listed for the three groups of cellulosics. Protein at 10-11% is high relative to other cellulosics. It is conjectured that the protein may form highly insoluble complexes during the high temperature pretreatment to hydrolyze the hemicellulose. There may, therefore, be some incentive to remove more of it with the juice by means of

rewetting during the pressing process. The ash content at 8.6% is also relatively high. The value of this ash as agricultural fertilizer remains to be determined. The exact content of the solubles likewise has not yet been determined.

Alternately, the fibrous fraction could be field-dried and combusted for production of electrical power. Bomb calorimeter tests have given a higher heating value of approximately 19,000 kJ/kg. At a yield of 9.45 t/ha, this would be approximately 50,000 kWh/ha, or if converted to electricity at an efficiency of 0.3, electrical energy of approximately 15 MWh/ha.

Conclusions

"Staging" of the area to be cut by starting early and spreading the first cutting over approximately 35-days, with repeat cuttings of any area at 35 day intervals, appeared to work well in 1995 and 1996. This strategy could allow harvesting and processing equipment to be used quite steadily, at near capacity, during the entire growing season. On the other hand, it should be recognized that the data presented is for only one set of unreplicated plots for two harvesting seasons. The measured yields are 125%-150% of those frequently reported. Less favorable weather, for example, could reduce yields and delay regrowth.

The high unit values and yields of certain juice products — e.g., soluble food-grade protein concentrates: ≅ 0.6 t/ha, particulate protein-xanthophyll concentrates ≅ 1.6 t/ha, industrially valuable enzymes ≅ 5-25 kg/ha — can make the economics of wet fractionation attractive. The potential high value of the juice products can, in a sense, "subsidize" the ligno-cellulosic fraction making it possible to market it in the neighborhood of $40/t dry matter.

The yield and composition of the fibrous fraction would allow over 4000 liters/ha of ethanol to be produced annually at 0.85 of the stoichiometric conversion. If converted to electrical energy at an efficiency of 0.3, the result would approximate 15 MWh/ha annually.

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Table 1. Composition (%) of the fibrous fraction obtained from maceration and juice expression of alfalfa (4 plots x 4 cuttings per plot).

	Cellulose	Hemicellulose	Lignin	Protein	Ash	Solubles (by difference)
Mean	33.05	17.48	7.77	10.94	9.28	21.48
Std. Dev.	4.40	3.44	1.67	1.75	2.00	3.91
Max.	43.38	24.40	12.97	15.56	16.38	28.32
Min.	24.04	12.52	4.83	8.13	6.81	14.51
n = 16 x 2 reps						
t/ha*	3.12	1.65	0.74	1.03	0.81	2.09

*Based on annual herbage yield of 13.5 t/ha and fibrous fraction = 0.7 x herbage = 9.45 t/ha.

Table 2. Potential ethanol production from alfalfa "fiber."

Material	t/ha	Stoichiometric Ratio	Efficiency	Ethanol yield (t/ha)
Cellulose	3.12	.568	.85	1.51
Hemicellulose	1.65	.581	.85	0.81
Solubles	2.09	.568	.85	1.01
		(assumed)	Total	3.37 t/ha = 4266 liters

Phytase From Transgenic Alfalfa for Supplementation of Poultry and Swine Rations

R.G. Koegel, S. Austin-Phillips, M.E. Cook and R.J. Straub

Introduction

Buildup of phosphorus in the environment and the resulting degradation of water resources is of mounting concern. Much of this buildup is traceable to human activities. Important among these is livestock production. Monogastric animals, such as poultry and swine, which can solubilize only a small fraction of the phosphorus in their grain-based rations while excreting the remainder, have come under increased scrutiny. Supplementation of inorganic phosphorus into rations to meet animal nutritional requirements exacerbates the problem.

Much of the phosphorus in grain is in the form of insoluble phytates. Researchers have shown that supplementing poultry and swine rations with the enzyme phytase can lead to solubilization of the phosphorus, thus eliminating the need for phosphorus supplementation and concurrently reducing the level of phosphorus in the excrement to approximately one-half of that normally experienced.

Because of relatively higher population and animal densities in western Europe, concern over phosphorus buildup has preceded that in the U.S. Accordingly, certain areas, like the Netherlands, have mandated limits on animal numbers and/or required the use of phytase in animal rations.

The enzyme phytase derived from *Aspergillus niger* has, to date, generally been produced in fermentation vats using genetically engineered microorganisms. It has been estimated that the cost of phytase supplementation with this material would be about three times the cost of conventional supplementation with dicalcium phosphate.

As an approach to reducing the cost of phytase production, a multi-disciplinary ARS-UW team at Madison, Wisconsin has produced transgenic alfalfa with the capability of expressing phytase. This phytase can be recovered with juice extracted from the herbage. Other constituents of the juice including xanthophyll, used to pigment egg yolks and broiler skin; high levels of dietary protein and various vitamins and minerals add to its value in rations. The use of whole alfalfa herbage, however, would not be desirable due to its high fiber content. Since phytase would potentially be needed in great quantities, but not in very pure or concentrated form, it is believed that the economic advantage of production in "plant bioreactors" such as alfalfa would be great. The advantage in capital costs is particularly great. Ideally, the cost of phytase supplementation should be competitive with the traditional dicalcium phosphate supplement, with the environmental benefits as an added incentive.

Methods

Through collaborative efforts with Dr. David Russell (Agracetus), we obtained *Agrobacterium tumifaciens* strains containing a derivative of the pBI binary vector in which the *Aspergillus niger* phytase gene had been placed under the control of either the CaMV 35S promoter or the *Arabidopsis thaliana* Rubisco small subunit (SSU) promoter. Both constructs also incorporated a signal peptide for targeting of the phytase enzyme to the apoplast. We also constructed an additional expression cassette utilizing the hybrid "MAC" promoter, which contains elements of both the CaMV 35S and *Agrobacterium* nopaline synthase promoters. This promoter was fused to the *A. niger* phytase gene (provided by Dr. Edward J. Mullaney, USDA), again incorporating a signal peptide for apoplast localization. This expression cassette was cloned into a pCGN binary vector and mobilized into *Agrobacterium*.

Transgenic plants were propagated by cuttings to generate a sufficient number of plants for juice

expression as described below. Whole juice was used in a preliminary feeding trial in which day old chicks were fed a grain-based diet amended with either dicalcium phosphate (at levels corresponding to 0, 33, 66, or 100% of NRC requirement) or alfalfa juice.

Results

In vitro transformation of both tobacco (W38) and alfalfa (RSY27) was accomplished with all three expression constructs. Significant levels of protein expression were obtained in all cases. Although plant-expressed phytase was underglycosylated in both tobacco and alfalfa (based on mobility on SDS-PAGE), the enzyme retained stability to high temperature (55 °C) and low pH (2.5). Comprehensive analysis of transgenic phytase-expressing plants is currently underway.

Of the transgenic alfalfa plants obtained, those transformed with the 35S promoter construct gave the highest levels of phytase expression, with several individual transgenic plants yielding phytase activity corresponding to 1 - 2% of total extracted protein.

During the 3 week trial (Table 1), chicks fed control diets lacking calcium phosphate either with or without control juice (expressed from non-transgenic RSY27) did not survive. While those chicks receiving phytase-amended feed (formulated at 400 units phytase per Kg feed) did not do as well as those receiving calcium phosphate, their relatively good performance indicated that the use of phytase-containing alfalfa juice is a very practical alternative to the addition of inorganic supplemental phosphate to the diet. It should be noted that the chicks with alfalfa juice in their diet received less calcium than those fed monocalcium phosphate. Furthermore, it is not known whether phytase was fed at the optimum level.

Plant-expressed phytase retained the stability observed for the *A. niger* enzyme. When either lyophilized or whole phytase-containing alfalfa juice was added to poultry feed at 400 units/Kg, no appreciable loss of activity was observed over 3 weeks of storage at room temperature (22 °C). In addition, we have verified that phytase in alfalfa juice (at 400 units/Kg) can efficiently release phosphate from phytase present in poultry feed in vitro.

Conclusions

Phytase was produced in transgenic alfalfa at concentrations of over 1½% of the soluble protein. Juice from this alfalfa proved to be effective in replacing inorganic phosphorus supplements in the diets of chicks.

Table 1. Chick feeding trial comparing inorganic phosphorus supplementation with phytase from alfalfa juice — corn/soybean diet. 25 chicks per treatment. November 1996.

Treatment	Week 1		Week2*		Week 3*	
Monocalcium phosphate						
(% P in diet)	Gain	Feed/gain	Gain	Feed/gain	Gain	Feed/gain
0%	**					
0.05%	83	1.167	245	1.365	504	1.495
0.10%	81	1.340	268	1.244	604	1.345
0.21%	87	1.345	304	1.357	597	1.515
Alfalfa juice						
No phytase	**					
400 IU Phytase/kg feed	86	1.336	244	1.560	492	1.431

*Numbers are cumulative from beginning of trial.

**Chicks euthanized after week 1 due to declining condition.

Research will continue in four areas:

1. Propagation of high-producing transformants to yield enough herbage for additional feeding trials.
2. Plant breeding to produce cultivars with good phytase yield, persistence, and high production characteristics.
3. Evaluation of alternative processes to transform juice into stable form(s) acceptable to the feed industry.
4. Additional feeding trials with both poultry and swine to determine optimum levels of phytase, stability over time, and reduction of phosphorus in feces.

A Lactic Acid Bacterial Strain to Improve Aerobic Stability of Silages

R.E. Muck

Introduction

Inoculants containing lactic acid bacteria are common additives used by farmers in the U.S. and other parts of the world in making silage. These products help guarantee a rapid and efficient fermentation of a crop in the silo. When inoculant bacteria dominate fermentation, lactic acid relative to other fermentation products increases, dry matter recovery is improved, and animal performance may be enhanced. However, these products have been found in many cases to make silages more susceptible to heating and spoilage by yeasts and molds once the silage is exposed to air. This has been particularly true in corn and small grain silages. The goal of this study was to determine if several strains of microorganisms found in aerobically stable silages might improve a silage's resistance to heating.

Methods

Similar trials were performed in two succeeding years. Whole plant corn was chopped with a forage harvester and ensiled in 15 cm ID by 60 cm long PVC pipe silos. One end of the pipe was sealed with a rubber cap — the other with black polyethylene sheeting secured by duct tape. There were two replicate silos for each of five treatments: control and four inoculant strains applied at approximately 10^5 microorganisms/g crop. Two strains were lactic acid bacteria, and two were yeasts, all of which were isolated from aerobically stable silages. The microorganisms were grown individually in batch culture and diluted so that application rates were 1 ml inoculant solution/50 g crop. The control was sprayed with distilled water at the same rate.

After a minimum of 90 days of storage, the silos were opened. Depth of the moldy layer underneath the plastic was measured and discarded. The remaining silage was removed by 15 cm units of depth so that there were four blocks of silage per silo. Each block was analyzed for pH, dry matter, fermentation products, and aerobic stability. Aerobic stability was assessed by the time required for the silage to heat 1 °C above ambient temperature when stored aerobically in styrofoam buckets.

Results and Discussion

In general, the top layer or layer closest to the black polyethylene sheeting was the least stable, and the lower three layers were of similar aerobic stability. Of the various treatments, only one provided substantial and consistent improvements in aerobic stability over the control. As shown in Table 1, this microorganism, *Lactobacillus buchneri* TY16, more than doubled the time until heating with the exception of the top layer in the first year. The TY16 silages in the second year did not heat over the course of the aerobic stability test (37 d of aerobic exposure). In both years, acetic acid was higher in the TY16 silages, and in the second year, significant levels of propionic acid were measured in the TY16 silages. This lactic acid bacteria is a heterofermentative microorganism and would be expected to boost acetic acid content, improving aerobic stability. Propionic acid is a stronger inhibitor of yeasts and molds than acetic acid. The mechanism of propionic acid production is not known at this time, and it is unclear if this is directly from the activity of TY16.

Conclusions

These results suggest that *Lactobacillus buchneri* TY16 is a very promising microorganism for improving the aerobic stability of silage. Current studies are in progress investigating various aspects important to the commercialization of this microorganism. These studies are investigating the appropriate rate of application relative to other strains in a potential product, stability of the microorganism, and effects of the

microorganism on animal performance. Such studies are being carried out in collaboration with Cargill, Inc. under a cooperative research and development agreement.

Table 1. Comparison of the characteristics of corn silages made with and without inoculation with *Lactobacillus buchneri* TY16.

	Year 1		Year 2	
	Control	TY16	Control	TY16
Moldy layer, cm	5.3	5.5	7.0	0.0
Time to heat, h				
Top layer	35	57	144	> 900
Lower layers	84	231	212	> 900
pH	3.84	3.86	3.85	4.36
Dry matter, %	33.1	32.8	31.3	29.7
Fermentation products				
Lactic acid*	5.68	5.57	6.50	1.35
Acetic scid*	1.59	2.81	1.64	5.87
Propionic acid*	0.00	0.00	0.01	0.46
Butyric scid*	0.00	0.00	0.00	0.00
Ethanol*	1.12	1.06	0.32	0.76
Aerobic microorganisms				
Yeasts+	2.4	3.6	< 2.0	< 2.0
Molds+	< 2.0	< 2.0	2.5	< 2.0
Acetic acid bacteria+	6.6	7.4	< 2.0	< 2.0

*% Dry matter; average of the lower three layers.

+Log_{10}(microorganisms/g silage); average of the lower three layers.

Ensiling of Potato Vines

R.E. Muck, Z.G. Weinberg, D.I. Rouse and B.R. Igl

Introduction

Potato vines are currently killed with herbicides before potatoes are harvested. However, the vines could be used as cattle feed if they could be properly stored. Furthermore, the harvesting of the vines would not only reduce herbicide use but also minimize the carryover of potato diseases and vectors in the field to the next potato crop. The most likely means of storing the vines is ensiling. The vines have a high moisture content, and the hills or ridges in the field on which potatoes are grown would make harvesting wilted vines difficult. The purpose of the current work was to find the best methods to preserve the potato vines by ensiling.

Methods

Experiment 1. Vines of 4 potato varieties were harvested with a flail forage harvester set at three different heights to obtain high, medium and low soil contamination levels. The chopped vines were ensiled in pint (~ 0.5 l) canning jars, alone or in combination with chopped alfalfa hay or barley grain at a ratio of 3 vines:1 amendment (w/w). Four replicates per treatment were ensiled. The silages were stored at room temperature for three months and then subjected to an aerobic stability test.

Experiment 2. A single variety (Russet Norkotah) of vines was hand harvested. Half of the vines were chopped fresh with a stationary chopper and ensiled in pint canning jars alone or in various combinations with chopped whole-plant corn (0:1, 1:1, 1:2 and 1:4 vines to corn, w/w). The other half of the vines were wilted in a greenhouse for 24 h and ensiled alone, inoculated with 10^5 lactic acid bacteria/g crop, and in mixtures with whole-plant corn at ratios of 0:1, 3:1, 1:1, 1:3 vines to corn. Three silos per treatment were opened after 1, 2, 6, 14 and 90 d of ensiling and analyzed. The 90 d silages were tested for aerobic stability.

Results and Discussion

Experiment 1. Analysis of the fresh vines (low soil) is given in Table 1. The pH values of the final silages were 5.6-6.2, 4.5-5.2 and 3.8-4.0 for vines alone and amended with hay and barley, respectively. The major microbial populations in the final silages were lactic acid bacteria, bacilli and acetic acid bacteria (10^8, 10^5 and 10^4-10^7 per gram, respectively). Yeast and mold populations were generally below detectable level. Soil level had no discernible effects on fermentation despite increasing ash contents to approximately 50% dry matter (DM) in the high soil contamination treatments. The silages with hay and barley had DM contents of 29.3 ± 0.7% and 28.8 ± 1.3%, respectively, and had pleasant aromas. In contrast, many of the vine-alone silages were noticeably clostridial. Over five days of aerobic exposure, the silages comprising vines only were the least stable with most replicates heating within that period. Several barley-amended replicates heated whereas the alfalfa hay-amended treatments were stable.

Experiment 2. Wilting the potato vines for 24 h in a greenhouse under good drying conditions increased DM contents only from 11.7% to 16.1%. Figures 1and 2 shows the changes in pH throughout the ensiling period. Silage pH increased after 1 to 2 weeks of fermentation in all of the vine-only treatments. In the unwilted vines, this was caused by clostridial fermentation. In the wilted vines, alone or inoculated, pH increased because of the fermentation of lactic acid to acetic acid, most likely by lactic acid bacteria. The mixtures of vines and corn resulted in high quality silages with pHs less than 4.5 except for the 3:1 mixture of wilted vines to corn. The silages comprising only vines or corn were the least stable upon aerobic exposure whereas none of the mixed silages heated over five days of aerobic exposure.

Conclusions

Potato vines have a potential to be used as feed because the high crude protein contents and low NDFs compensate for high ash contents. However, the harvested vines alone were consistently too low in DM and sugar contents to achieve a sufficiently low pH to prevent secondary fermentation. Also, silage effluent would be a significant concern in such unamended silages. Wilting the vines for 24 h under good drying conditions in a greenhouse made only modest increases in DM content and still did not permit adequate preservation.

High quality silages of potato vines were obtained only in mixtures with drier crops, which also dilute ash levels. All three amendments (alfalfa hay, barley grain and whole-plant corn) produced good silages. Of the three amendments, barley grain produced the lowest pH, but these silages were the least aerobically stable of the amended silages. The best option would be to co-ensile the potato vines along with a crop which is harvested at the same time, such as early maturing corn varieties. This would minimize the labor for harvesting and storage. Mixing with corn would also have two advantages: increasing the low crude protein content of the corn silage and improving its aerobic stability.

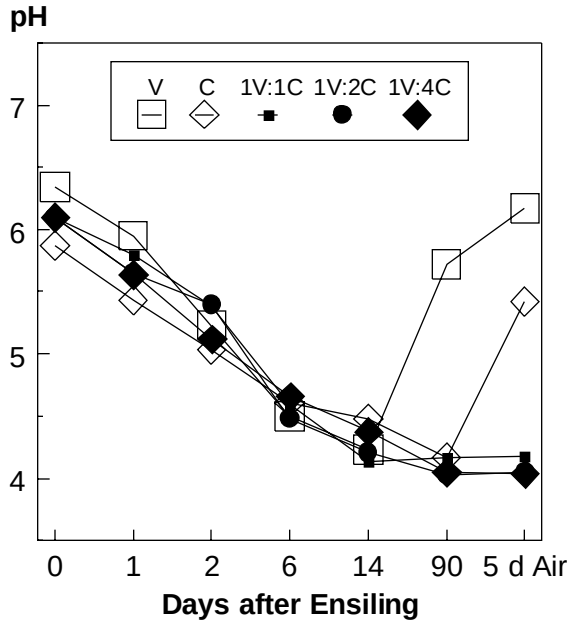

Figure 1. Changes in pH of fresh potato vine silages, alone or amended with whole-plant corn, during ensiling and after 5 d of aerobic exposure. V - vines, C - corn.

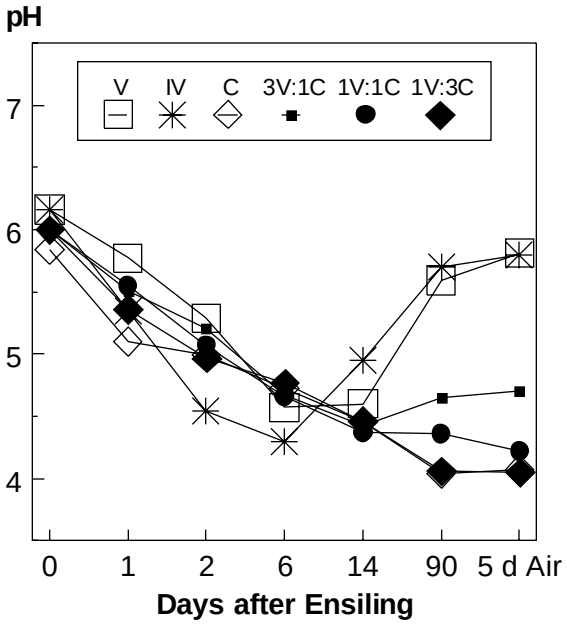

Figure 2. Changes in pH of wilted potato vine silages, alone or amended with whole-plant corn, during ensiling and after 5 d of aerobic exposure. V - vines, C - corn, I - inoculated.

Table 1. Analysis (% DM except as noted) of the fresh potato vines (low soil level) in Experiment 1.

Variety	DM[1]	Ash	NDF	ADF	ADL	CP	WSC[2]	BC[3]	LAB[4]
Norkotah	13.2	26.2	34.8	29.4	5.1	19.4	5.8	376	4.1
Norland	12.6	29.3	31.9	23.5	4.8	22.1	5.2	565	3.7
WI 1005	10.7	22.0	35.9	27.5	4.4	26.0	7.0	380	3.9
WI 1099	12.2	33.0	28.6	24.5	3.5	22.4	4.5	415	3.3

[1]%

[2]Water soluble carbohydrates

[3]Buffering capacity, meq/kg DM

[4]Lactic acid bacteria, $\log_{10}$ CFU/g vines

Proteolysis in Different Forage Silages

R.E. Muck, D.R. Mertens and R.P. Walgenbach

Introduction

One of the most significant processes occurring during ensiling is proteolysis or the enzymatic breakdown of proteins to soluble nonprotein nitrogen (NPN) forms such as peptides, free amino acids and ammonia. This process occurs in the first few days of ensiling and may result in up to 85% of the crude protein in alfalfa silage being soluble NPN. This loss of true protein, particularly in silages fed to high-producing dairy cows, may significantly reduce the efficiency of nitrogen utilization by ruminants. Although considerable information is available concerning proteolysis in alfalfa and red clover, much less is known about other forage silages. The objectives of this study were to: 1) investigate the effects of maturity and dry matter (DM) content on proteolysis during ensiling in seven crops (alfalfa, red clover, orchardgrass, wheat, barley, sorghum-sudan grass and corn), and 2) determine if proteolysis can be reasonably predicted by simple means available to commercial laboratories.

Methods

Silages of different forages were made over two years. Each forage was harvested in primary growth and at least one regrowth period per year except for small grains and corn (only primary growth). Harvesting began when a forage reached a late vegetative stage and continued through at least late flower development. This was done on a weekly basis the first year and biweekly basis the second year. One portion of mowed forage was chopped in a stationary chopper and ensiled immediately. Except for corn, four other portions were weighed onto screens and dried in the greenhouse to 35, 45, 55 and 85% DM. The 35 to 55% DM portions were chopped and ensiled upon reaching the desired DM content. The 85% DM forage was stored dry to represent hay. Ensiling was done in pint (473 ml) canning jars. Prior to ensiling, all crops were inoculated at a rate of 10^4 bacteria/g crop with a commercial inoculant containing *Lactobacillus plantarum* and *Pediococcus cerevisiae* to guarantee a minimum level of lactic acid bacteria for fermentation. Silos were stored at room temperature for 30 days. Unensiled forages and silages were analyzed for DM, pH, crude protein, and soluble NPN. Silages were analyzed for fermentation products.

Results and Discussion

In the first year, much of the growing season was cool and very wet, whereas in the second year the first half of the growing season was warm with generally below normal precipitation. This variation in weather conditions produced differences in fermentation. Most silages were well preserved, but out of 600 silages, 12 had significant levels of butyric acid in the wet year and only 2 in the dry year. Also silage pHs, particularly in the legume silages, were higher and had a greater range in the wet year compared with the dry year. Most likely, the wet year produced forages of lower sugar content and higher buffering capacity.

In spite of fermentation differences, proteolysis did not appear to be affected by year. Soluble NPN (% DM) for all crops is shown in Figure 1. What was surprising was the continuum among

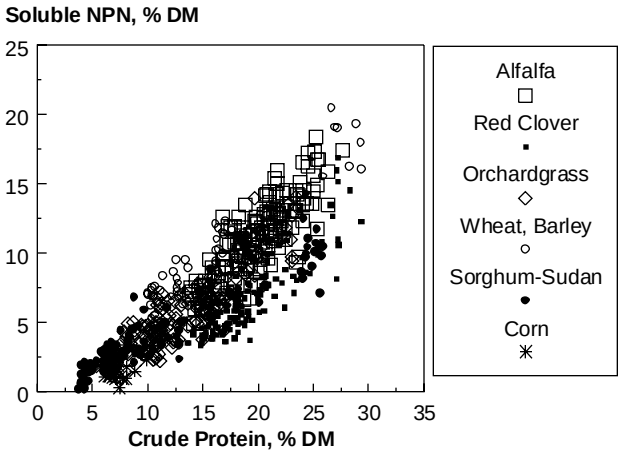

Figure 1. Soluble NPN as a function of crude protein content for all silages in both years.

crops with the exception of red clover and possibly sorghum-sudan grass. The lower NPN levels in red clover were anticipated based on earlier work at the Center, implicating the action of polyphenol oxidases. It is not known what may be affecting proteolytic activity in sorghum-sudan grass.

Stepwise regression was used to determine which factors would be useful in predicting soluble NPN (% DM basis). For all crops, crude protein and DM contents were the most important factors (Table 1). In some cases, silage pH significantly improved the prediction of soluble NPN. Excluding red clover and sorghum-sudan grass, a single regression using crude protein and DM contents as independent variables could explain 87% of the variation in soluble NPN across the other crops. The equations are currently being used to predict soluble NPN from silages (both laboratory and field-scale silages) reported in published studies. Preliminary results indicate that the equations are reasonably predicting soluble NPN in these other studies.

Conclusions

Overall, these results suggest that soluble NPN can be estimated from parameters that are routinely provided by forage testing laboratories. This should help forage testing laboratories to more accurately estimate the feeding value of silages for farmers. Further validation work is needed to prove that this concept in estimating soluble NPN is sound and to establish the level of error associated with the prediction equations.

Table 1. Regression equations for soluble NPN (% DM basis) in forage silages.

Crop	Number of parameters	Equation[+]	R^2, %
Alfalfa	2	-0.840 - 0.0629 DM + 0.722 CP	77.1
	3	-0.952 - 0.0633 DM + 0.0364 PH + 0.720 CP	77.1
Red Clover	2	-3.185 - 0.0590 DM + 0.640 CP	77.3
	3	-8.242 - 0.0823 DM + 1.590 PH + 0.568 CP	80.0
Orchardgrass	2	-0.336 - 0.0326 DM + 0.553 CP	78.8
	3	2.065 - 0.0235 DM - 0.605 PH + 0.553 CP	79.2
Wheat, Barley	2	1.000 - 0.0570 DM + 0.684 CP	89.5
	3	-3.827 - 0.0895 DM + 1.596 PH + 0.609 CP	90.3
Sorghum-sudan	2	1.670 - 0.0547 DM + 0.432 CP	89.3
	3	-2.520 - 0.718 DM + 1.229 PH + 0.399 CP	90.2
Corn	2	1.250 - 0.0365 DM + 0.299 CP	59.6
	3	-0.564 - 0.0450 DM + 0.532 PH + 0.300 CP	61.2
Combined*	2	-0.947 - 0.0506 DM + 0.699 CP	86.6
	3	-1.824 - 0.0548 DM + 0.267 PH + 0.686 CP	86.7

[+]DM - dry matter content, %; PH - pH; CP - crude protein, % DM

*Combined - all crop data except red clover, sorghum-sudan grass

Round Bale Silage Storage Losses of Ryegrass and Legume-Grass Forages

R.L. Huhnke, R.E. Muck and M.E. Payton

Introduction

Round bale silage offers producers another option for harvesting and storing high quality forage. Even though the factors influencing silage quality are well documented, producers often harvest and wrap forages outside the limits considered acceptable for adequate fermentation and stable storage. This is especially true for moisture content, where the recommended range for successful ensiling is approximately 50 to 70% on a wet basis. Moisture contents below 50% are discouraged because of the increased likelihood of heating and respiration losses. The objective of this research was to measure silage quality and dry matter (DM) losses in round bale silages wrapped at moisture contents at and below recommended levels.

Methods

The study was carried out at four farms in eastern Oklahoma. First cutting forages (ryegrass or grass-clover mixtures) were harvested and ensiled. Grass ranged from late bloom to dough stage while clovers ranged from mid to late bloom. Bales were formed using a Vermeer Model 504I baler and were approximately 1.2 m in diameter and 1.2 m in width. At least 5 bales were made during three to four baling periods over two days at each location, giving a range of moisture contents. Bales were cored for analysis of moisture content and quality prior to wrapping. Bales were wrapped within two hours of being formed with white stretch film. At least six layers of film were used on each bale. Bales were weighed before being placed into storage on an exposed site at each location. Each bale was spaced at least 0.5 m apart from adjacent bales. After a minimum of six months storage, bales were weighed and cored at mid-height on each side. Samples were taken from the outer 10 cm and from 10 to 23 cm, representing approximately one third of bale volume. Samples were analyzed for moisture, pH, crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), total digestible nutrients and fermentation products.

Results and Discussion

Across the four locations, moisture content at ensiling varied from 25.3% to 69.1%, wet basis, and dry densities ranged from 101 to 203 kg/m^3. After at least six months of storage, average weight losses were small. For moisture contents above 50%, average weight losses for the four locations ranged from 0.8 to 2.0%. Lower weight losses were measured in the drier bales, 0.0 to 0.7%. Significant increases in CP and ADF occurred between the initial forage samples and final silages whereas NDF was unchanged except at the McAlester farm (Table 1). The relatively constant NDFs most likely were caused by the enzymatic breakdown of hemicellulose during ensiling offsetting the increase in concentration from DM losses. Changes in these quality parameters were not significantly affected by moisture content at any of the locations except for NDF at the McAlester farm. Assuming no loss or formation of CP and ADF in the bales, estimated DM losses across the four locations ranged from 5.6 to 9.1% and 2.4 to 11.7% based on CP and ADF contents, respectively. These estimated losses are low relative to those expected in traditional upright and bunker silos. However, our results are in agreement with those reported by other wrapped bale studies with various forages in other parts of the world.

Bale moisture content did affect fermentation. In general, silage pHs increased and lactic and acetic acid contents decreased with decreasing moisture content. Silage pHs tended to be higher and fermentation product levels lower than typically observed in silages from upright or bunker silos. Nevertheless, good preservation was observed in most bales. Only four bales

across the four locations had detectable levels of butyric acid, an indicator of poor fermentation, and none of these bales had butyric acid contents high enough to affect intake. Of the 312 samples analyzed, 18% had pH values above 6.5, indicating spoilage. Most of these samples were in the outer core samples. The proportion above pH 6.5 was relatively constant across samples having moisture contents less than 65%. Thus bales ensiled at moisture contents that would normally be considered too dry (< 50%) were apparently preserved as well as bales ensiled at recommended moistures (50 to 70%).

Conclusions

Grass and grass-legume silages made at low moisture content (< 50%) in wrapped round bales had similar changes in quality (CP, NDF, ADF) as silage made at higher, recommended moisture contents. Fermentation was reduced in low moisture bale silages, but spoilage and estimated DM losses were low and similar over a wide range of moisture contents. Consequently, the results of this study show that forages at moisture contents above that considered safe for dry storage (> 20%) but less than that acceptable for well-preserved silage (< 50%) can be preserved well in wrapped round bales provided that the integrity of the plastic film is maintained.

Table 1. Average bale quality at the four sites.

	CP (% DM)			ADF (% DM)			NDF (% DM)		
		Outer Layer, cm			Outer Layer, cm			Outer Layer, cm	
Location	Initial	0-10	10-23	Initial	0-10	10-23	Initial	0-10	10-23
McAlester	11.4	11.9	12.1*	39.6	41.3*	40.2	69.4	67.1*	65.7*
Hugo	13.5	14.8*	14.7*	36.7	42.6*	40.2*	60.9	62.1	59.1
Stillwater	11.8	13.3*	12.6*	37.5	40.1*	40.1*	55.5	55.2	55.1
Haskell	13.0	14.0*	13.6*	35.2	37.3*	37.3*	57.8	58.7	58.1

*Silage characteristic is significantly different ($P < 0.05$) from that in the initial forage.

Economics of Alternative Silage Systems

C.A. Rotz

Introduction

Concrete tower silos have been used on dairy farms for many years. Bunker silos are becoming more popular, particularly on larger farms, because they offer more rapid filling and emptying. Many bunkers are not covered though, which causes greater feed losses. Another option is bagged silage where silage is pressed and sealed in large bags. Most recently, baled silage has gained some popularity. Large round bales of wet hay are wrapped in plastic where they ferment. Quantifying the costs and benefits of alternative storage methods is not easy. Technology that performs well under one set of crop and weather conditions may not perform well at other times. Long term studies are needed over a wide range of conditions. Models such as DAFOSYM, developed and validated with limited experimental work, can be used to study system performance over many years of weather. Many alternative dairy systems have been modeled with DAFOSYM to determine their value to producers.

Materials and Methods

DAFOSYM is a simulation model of crop production and feed use on dairy farms and the return of manure nutrients to the land. This dairy forage system is simulated over many years of weather to determine long term performance and economics of alternative technologies and/or management strategies. By modeling several options on the same representative farms, those that provide maximum farm production or profit are determined. As an example of the use of the program, silage systems using either stave silos, uncovered bunkers, silage bags, or bale silage were compared. This was not intended to be an extensive comparison of these systems, but simply an example of how these storage systems compare on a typical farm.

The farm represented a typical farm in southern Michigan with 100 high-producing Holstein cows plus 85 replacement heifers. Feed rations were determined for two groups of heifers, a dry cow group, and three groups of lactating animals. A mobile mixing wagon was used to prepare total mixed rations for each animal group. Round bales of hay were self-fed and available as needed.

Essentially all forage and grain feeds required by the herd were produced from 120 acres of alfalfa and 150 acres of corn. Alfalfa was harvested using a four cutting harvest strategy with the first two cuttings harvested at a bud stage of development and the last two harvested in early bloom. Harvests began within 5 days of May 30, July 6, and August 20 for the first three cuttings and on October 15 for the fourth cutting. First, third, and fourth cuttings were harvested as wilted silage, and second cutting was baled in large round bales. Corn was harvested as silage and high moisture grain to fill the available silos, and additional corn was harvested as dry grain.

Results and Discussion

The type of storage affects harvest rates, forage losses, the nutritive value of feeds produced, and animal performance (Table 1). Greater loss in bunker silos reduces the alfalfa and corn silages available as feed. Nutritive changes affect the corn and protein supplements required to meet the herd energy and protein requirements. Nutritive loss in bunker silos causes a small drop in milk production, and the lower digestibility of this silage leads to slightly more manure to handle. Nutritive changes in bale silage influenced the nutritive content in manure which caused a slight increase in fertilizer use.

The silage system selected affects machinery use, production costs, and farm profitability. With bunker silos, harvest and feeding rates are a little higher which reduces machinery operating costs and the use of fuel and electricity. Storage costs are lowest for silage bags priced at $5/ton

DM of silage and highest for bales wrapped with plastic costing $20/ton DM of silage. The two bunker silos (40 ft. x 140 ft., $45,000 each) had a higher initial cost than the four stave silos (18 ft. x 70 ft., $19,500 each) which led to slightly higher storage costs. Labor cost was a little higher for the bunker silo due to an extra person needed to operate the packing tractor. Overall, the annual net return or profit of the farm was $13,500 greater using the bag silage system compared to stave silos. Use of uncovered bunker silos reduced net return by $14,500 per year. The bale silage system reduced farm net return $2,000 per year below that of the stave silo system.

Conclusion

The most economical silage system for the 100 cow dairy farm was a bagged silage method. Use of either stave silos or wrapped bale silage provided similar farm profits which were substantially less than those of bagged silage. The least profitable storage method was an uncovered bunker silo.

Table 1. Effects of silage storage method on feed use, annual costs, and annual net return of a 100 cow (270 acre) dairy farm producing corn and alfalfa silages.

Production or cost parameter	Units	Stave silos	Uncovered bunkers	Silage bags	Silage bales
Alfalfa hay production	ton DM	143	144	143	144
Alfalfa silage production	ton DM	345	302	362	341
Corn silage production	ton DM	291	277	308	290
High moisture corn production	ton DM	160	160	160	160
Corn grain production	ton DM	54	55	55	54
Alfalfa purchased (sold)	ton DM	(14)	31	(43)	(13)
Corn grain purchased (sold)	ton DM	29	47	8	36
Protein supplements purchased	ton DM	47	42	58	42
Average milk production	lb/cow	20,973	19,912	21,355	20,882
Manure production	ton	6,966	7,249	6,786	6,999
Field and feeding machinery cost	$	49,134	44,939	47,317	46,596
Fuel and electric cost	$	6,330	5,912	5,966	5,975
Feed and machinery storage cost	$	22,164	23,527	18,660	26,295
Labor cost	$	35,288	36,602	35,178	35,077
Seed, fertilizer, and chemical cost	$	13,935	13,991	13,873	14,260
Corn drying cost	$	1,019	1,021	1,021	1,019
Purchased feed and bedding cost	$	26,992	29,521	27,278	26,485
Animal and milking facilities cost	$	35,261	35,261	35,261	35,261
Livestock expenses	$	23,800	23,800	23,800	23,800
Milk hauling and marketing fees	$	18,501	17,565	18,838	18,421
Property tax	$	4,924	4,994	4,554	4,739
Total production cost	$	237,348	237,133	231,746	237,926
Milk, feed, and animal sale income	$	294,906	279,554	302,328	293,434
Net return to management	$	57,558	42,421	70,582	55,508

Using Molecular Modeling to Predict Spectral Characteristics of Peroxidase Products

R.D. Hatfield

Introduction

Determining spectral changes when reactants are converted to products is a convenient and sensitive method of monitoring the progress of reactions. It is useful to know ahead of time the spectral characteristics of the anticipated products. This is not always possible as the products may not be commercially available and/or spectral information is unknown. Molecular modeling provides a potential means of predicting the spectral characteristics of compounds that may be formed during a reaction (in some cases multiple products might actually be formed). To test this hypothesis, CAChe, a molecular modeling program, was used to predict the spectral characteristics of ethyl ferulate, typical ferulate dehydrodimers, sinapyl alcohol, and potential cross reaction products of sinapyl alcohol. Authentic samples of ethyl ferulate and sinapyl alcohol were available for verification of the predicted electronic spectra.

Materials and Methods

The CAChe system from Oxford Molecular Group was used to build models of predicted structures of dimers formed from sinapyl alcohol and ethyl ferulate. Molecular structures were optimized using MM2 molecular mechanic parameters, and ZINDO (Zerner's Intermediate Neglect of Differential Overlap) program was used to compute spectroscopic properties (electronic spectra) of the molecules.

Results and Discussion

Ethyl ferulate dehydrodimers, 8-5 (1), 8-*O*-4 (2), 5-5 (3), and 8-8 (4) (Fig. 1), were built using the CAChe molecular modeling program and all structures optimized for lowest energy using the

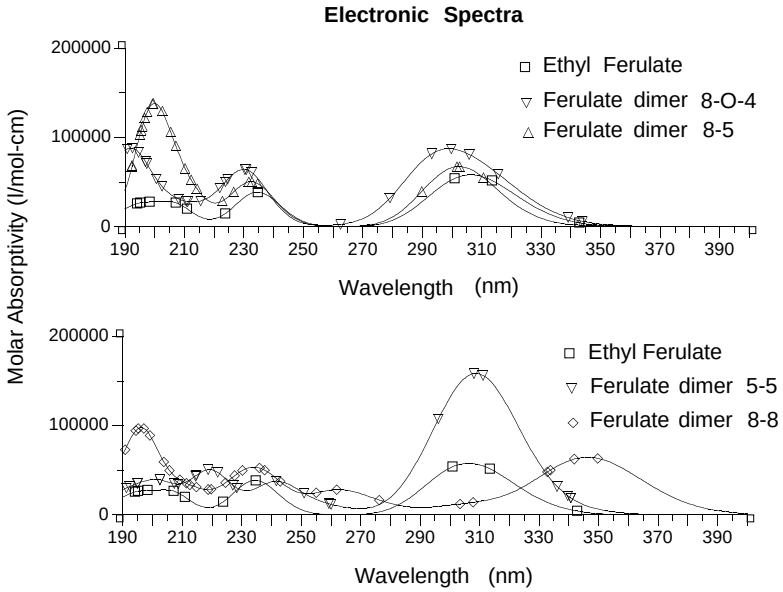

Figure 1. Ferulate dehydrodimers analyzed for spectral characteristics using the CAChe Molecular Modeling Program. Coupling products of sinapyl alcohol modeled with CAChe.

MM2 parameters. Predicted electronic spectra for the compounds are given in Fig 2. Although these predictions were based on molecules in a null environment having no other chemical interactions, the predicted maximum at 300 nm (Fig. 2) was similar to that observed for ethyl ferulate in an organic solvent such as methanol (maximum at 310 nm). The predicted spectra for the ferulate dimers show slight changes in the maximum around 300 nm except for the 8-8 dimer that has a significant shift to a longer wavelength (345 nm). These predicted values are consistent with observed spectra of ethyl ferulate dimers. Although the spectral region from 190 to 240 would be much more diagnostic for the ferulate dimers, this region is of little practical use in monitoring cross coupling reactions mediated by peroxidases due to the strong absorbance displayed by most buffering systems in this region.

Electronic spectral predictions for sinapyl alcohol and two potential cross-coupling products are shown in Fig. 3. In both cases the dimers have significantly different spectral properties from the sinapyl alcohol. We have not been able to obtain actual spectra for the two dimer products, but the predicted spectra for sinapyl alcohol is close to the observed spectra (maximum at 270 nm). From monitoring peroxidase mediated coupling reactions of sinapyl alcohol, there is a significant loss of the molar absorptivity maximum at 270 nm. Again the region from 190 to 230 nm would be diagnostic but is unavailable due to interference from buffers and solvents used to obtain the electronic spectra.

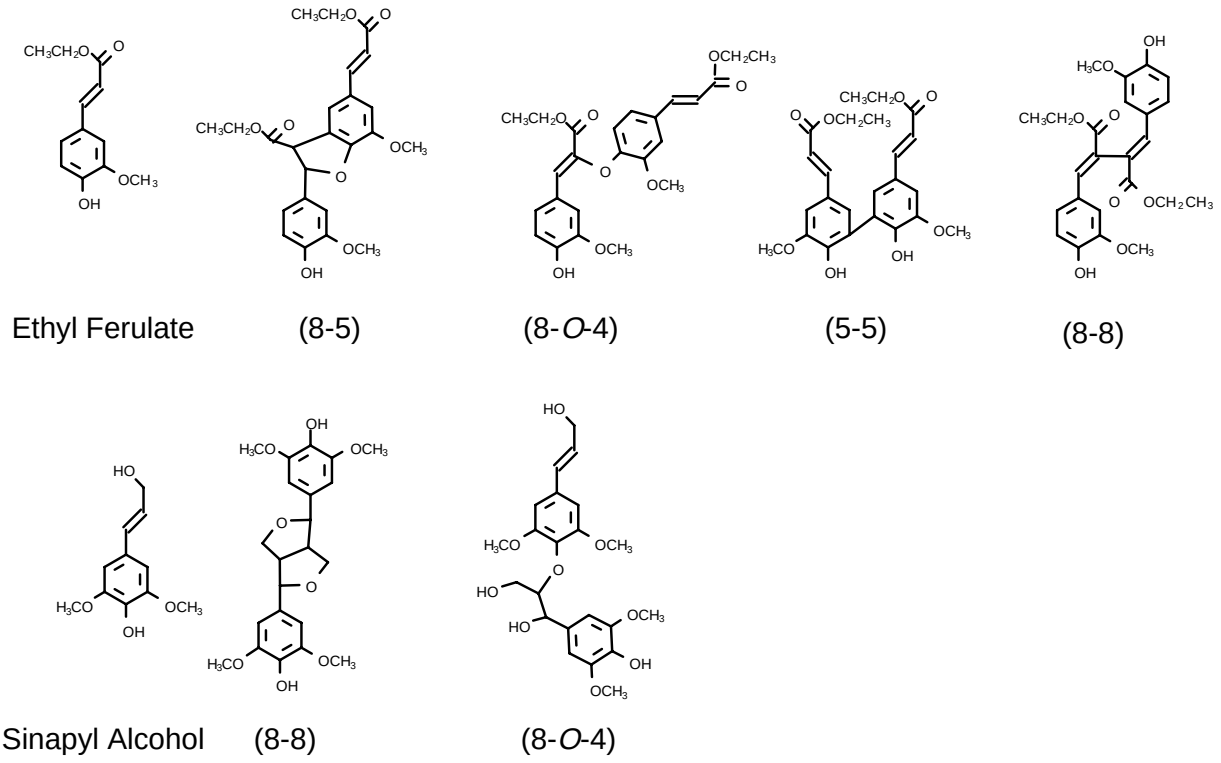

Figure 2. Electronic spectra predicted for ferulate dimers.

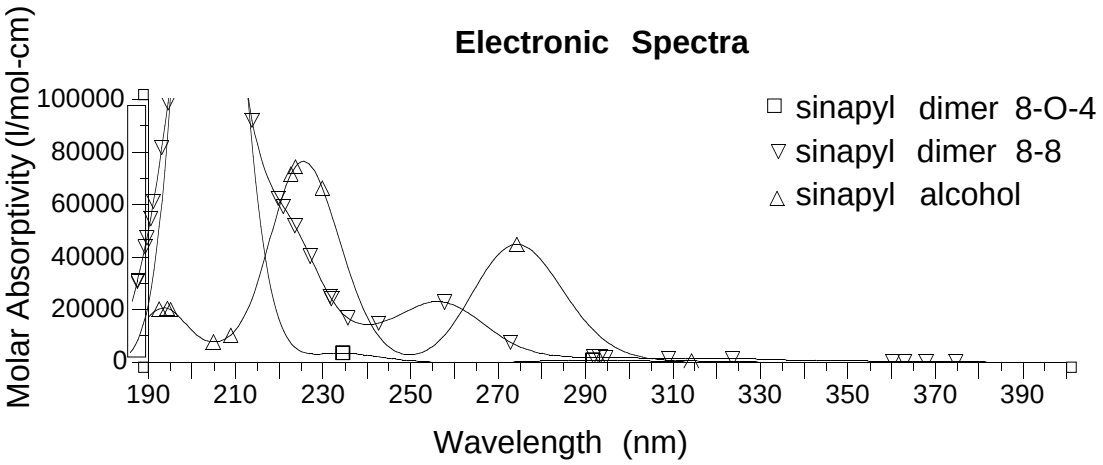

Figure 3. Electronic spectra predicted for sinapyl alcohol dimers.

Conclusion

Although it is not possible with this program to predict the precise electronic spectra, due to solvent influences, the predictions in a null environment are close to observed values. This is a useful tool to predict electronic spectra of small molecules providing insight into possible changes during certain types of reactions.

Impact

Development of spectral assays for monitoring reactions allows us greater flexibility in characterizing enzyme properties on small amounts of material. As our knowledge base increases, we are better able to understand and predict metabolic changes in plant cell wall chemistry/biochemistry leading to potential methods for improving forage utilization.

Formation of Intramolecular Ferulate Dehydrodimers: A Molecular Modeling Approach

R.D. Hatfield and J. Ralph

Introduction

It is becoming clear that the formation of ferulate dimers plays a crucial role in the structure and function of grass cell walls. With improved techniques for the detection of all the dehydrodimers, researchers are finding significantly higher amounts of wall cross-linking than previously assumed. If all of the ferulate dimers are intermolecular (between two polysaccharide chains) in nature, then there would be a significant impact on wall structure. If, on the other hand, intramolecular dimers (between two ferulates on the same polysaccharide chain) were formed, the impact on wall structure would be diminished. The nature of ferulate dimers attachment cannot be elucidated by normal solvolysis techniques used to analyze ferulates in walls. Release of the ferulate dimers by alkaline hydrolysis eliminates all structural information about how they were once attached within the wall. One approach is to digest cross-linked walls with hydrolytic enzymes and look for xylose oligosaccharides that have both ends of a ferulate dimer attached. This would be a tedious process. Furthermore, one may well be seeking something that does not exist. As an alternative, we have used molecular modeling to predict the likelihood of intramolecular formation of ferulate dimers.

Materials and Methods

The CAChe system from Oxford Molecular Group was used to build carbohydrate models containing two side chains of ferulated arabinose. Molecular structures were optimized to the lowest energy using MM2 molecular mechanic parameters. The initial structure contained 15 xylan residues with the ferulated arabinose residues on xylose 3 and 12 (see Fig. 1); this was considered the base structure. Experimental structures consisted of moving the side chain from xylose 12 to 4, minimizing this structure and coupling the two ferulates by 8-*O*-4, 8-5, 5-5 or 8-8 linkages and repeating the minimization. This cycle was repeated with the second side chain on xylose 5, 6, 7, then 8. The xylan backbone was either locked in its initial energy configuration or allowed to relax during the minimization procedures. If any structure gave a higher energy than the base molecule, the resulting minimized structure was tweeked by altering the structure and repeating the minimization. This prevented the molecule from becoming stuck in a local minimum that would not reflect the true minimized optimum structure. For some structures which appeared to produce low energy optimum structures with an intramolecular diferulate linkage, energy maps were constructed of major dihedral angles to determine if the molecule would naturally reach this configuration.

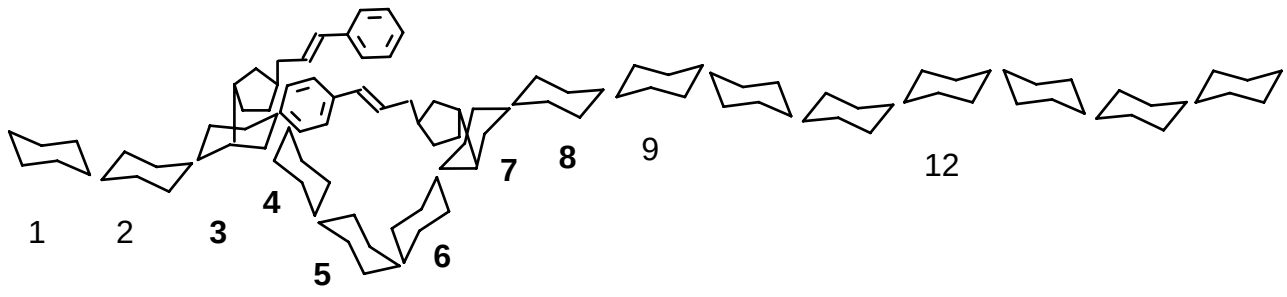

Figure 1. Schematic representation of a xylan fragment used in molecular modeling experiments. The left xylose residue is the non-reducing end of the molecule. Specific structural details have been left off to simplify the diagram. Numbers at the bottom of the diagram represent the numerical order of xylose residues used in the modeling experiments.

Results and Discussion

All structural configurations in which the xylan was allowed to fully relax produced energy minimizations nearly the same as the starting configuration before coupling the ferulates (any energy difference less than 20 Kcal was considered not to be an unfavorable energy configuration). However, with this set of parameters, formation of dehydrodimers at position 3-7 and 3-8 resulted in a buckling of the xylan backbone in order to achieve a low energy structure. Even the 8-8 dimer at position 3-6 resulted in some distortion of the backbone (Fig. 2). As might be expected, when the xylan backbone was held rigid, no ferulate dimers could produce a low energy configuration at positions 3-7 or 3-8, except for the 5-5 dimer at position 3-7. Our interpretation of these results is that, if ferulate monomers are attached to a xylan backbone with more than 3 xylose residues between them, it would not be possible to form intramolecular dimers. This is based on experimental evidence which suggests xylans prefer to stay in rigid rod type configuration with a three fold screw axis (no abnormal flexing to achieve coupling).

The one exception was the formation of the 5-5 dimer at position 3-7. This particular arrangement of ferulate monomers positions them in a configuration that is conducive to 5-5 coupling. This is of interest in that we cannot produce 5-5 coupled dimers using model compounds such as ethyl ferulate and wall peroxidases in free solution. Perhaps the formation of 5-5 dimers within wall matrices requires an exact positioning of the ferulate monomers.

For ferulate monomers in positions 3-4, 3-5 and 3-6, there is the potential for formation of dimers as long as the individual molecules can freely rotate around the axis of the xylan backbone. To check this possibility we have generated energy maps of the major dihedral angle changes that must occur to allow dimer formation. In most cases, it appears that sufficient energy barriers exist to prevent them from achieving the proper orientation necessary for coupling to form any of the dimers. The only configuration that appears feasible would be the 8-*O*-4 or 8-5 dimers which are in position 3-4. Ferulates in this position overlap sufficiently to allow coupling with minimum rotation along the xylan backbone axis.

Conclusions

Based on the modeling predictions combined with experimental evidence, intramolecular formation of ferulate dimers would appear to be restricted to positions 3-4 (dimers 8-*O*-4 and 8-5) or positions 3-7 (5-5 dimer). The latter is especially interesting since it may explain the unexpected preponderance of the 5-5 dimers in grasses.

Impact

Increased understanding of cell wall cross-linking chemistry affected by dimerization of ferulates will aid in the development of strategies for improving forage plants. These strategies may include genetic selection for or against specific traits, molecular modification of enzyme activity, or development of post-harvest treatments (specialized hydrolytic enzymes) to increase wall degradation.

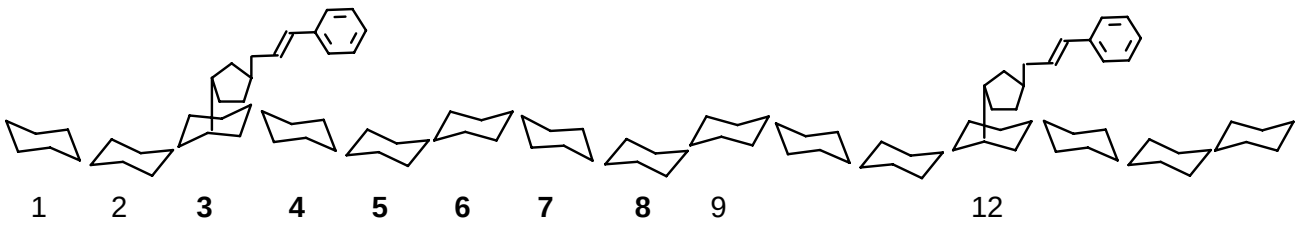

Figure 2. Schematic representation of a distorted xylan backbone when ferulate coupling is attempted with 4 (3-7) or more xylose residues between the ferulates.

Use of Calorimetry to Verify Lignin Concentration Estimates

H.G. Jung and V.H. Varel

Introduction

Lignin is a cell-wall polymer derived from free-radical reactions of hydroxycinnamyl alcohols and other cell-wall constituents. Lignin has been implicated as the primary component of cell walls that limits forage cell-wall digestibility by ruminants. A major difficulty in studying the role of lignin in cell-wall digestibility has been that a definitive molecular structure cannot be drawn for lignin, and all lignin concentration estimates are purely empirical, based on the particular method of analysis chosen. As a result, lignin concentration estimates vary widely among methods. Acid detergent lignin (ADL) employing 12 *M* H_2SO_4 is the most commonly used method in animal science and agronomy. We have previously shown that Klason lignin, which gives lignin concentration estimates two to five times higher than ADL, is not significantly contaminated with protein or carbohydrate and is similar to ADL in molecular composition. We believe the ADL method underestimates lignin concentration due to loss of acid-soluble lignin in the acid detergent step of the procedure. Calorimetry data are presented which verify that ADL does not account for all of the plant lignin and Klason lignin does not over-estimate lignin concentration.

Materials and Methods

A diverse group of 10 forage samples was used in this study. All forages had been dried and ground to pass a 1-mm screen in a cyclone mill. These forages were analyzed for total carbohydrate by a two-stage acid hydrolysis with neutral sugars quantified by GLC and uronic acids with colorimetry. Protein was determined as total N x 6.25. Lipids were analyzed by extraction with ether. Ash content of all samples was determined by combustion at 450 °C. Klason lignin was determined as the ash-free residue from the two-stage acid hydrolysis. Acid detergent lignin was determined by sequential detergent analysis. Bomb calorimetry was used to determine the gross energy (GE) of all forage samples. A calculated GE value was derived by applying published GE values for the forage chemical components to the measured concentrations of those components:

calculated GE = (protein x 5700 kcal/kg) + (carbohydrate x 4000 kcal/kg) + (lipid x 9500 kcal/kg) + (lignin x 8000 kcal/kg).

These calculations were done for ADL and Klason lignin concentration estimates. The calculated GE values were then compared to the corresponding measured GE of the forages.

Results and Discussion

The forages varied widely in chemical composition and Klason lignin values were, as expected, much greater than corresponding ADL values (Table 1). Measured concentrations of protein, lipid, and ash were similar to published values for these types of forages. Gross energy values of these forages determined by bomb calorimetry (Table 2) were also similar to expected levels. When GE was calculated from the analyzed composition of the forages, use of Klason lignin resulted in GE recoveries (as a percent of the measured GE) averaging 93.1% whereas a similar calculation of GE based on ADL resulted in an average recovery of only 75.8% (Table 2).

Because lignin concentration estimates were the only element of the calculation that changed, this difference in recovery between lignin methods was expected. However, if Klason lignin indeed overestimates lignin concentration, then the calculated GE should have been greater than 100% of measured GE. The facts that Klason lignin allowed a better accounting for measured GE than ADL and that this GE recovery was less than 100%, indicate Klason lignin is a more accurate quantitative measure of lignin concentration. The lack of complete recovery of

the measured GE in the calculation can be attributed to the incomplete recovery of sample dry matter by the component analyses. Using Klason lignin, dry matter recoveries ranged from 84 to 93%. If we assume all non-recovered GE by the component calculation is carbohydrate (the least energy dense forage component), then dry matter recoveries using Klason lignin increase (94 to 100%). We have reason to believe carbohydrate recoveries were incomplete because there were unidentified peaks on the neutral sugar chromatograms. We are investigating the source of these peaks.

Table 1. Chemical composition of the forage samples.

Sample	Lignin Protein	Carbohydrate	Lipid	Ash	ADL	Klason
			% DM			
Alfalfa	21.3	44.5	1.8	8.8	5.8	15.0
Alfalfa	16.2	46.1	2.3	10.6	5.9	13.6
Kura clover	21.1	41.2	4.0	8.9	2.1	8.4
Annual Medic	25.9	38.5	2.9	14.3	2.6	9.3
Corn silage	7.1	63.3	3.6	5.0	1.4	12.2
Corn silage	11.1	59.6	4.2	7.5	1.4	10.0
Orchardgrass	14.2	42.9	4.4	11.4	2.5	12.6
Bromegrass	11.6	52.9	1.6	9.0	3.8	15.5
Switchgrass	9.3	51.2	1.3	8.3	2.4	14.2
Oat straw	4.2	60.4	0.6	10.7	4.7	16.6

Table 2. Percent of measured forage gross energy accounted for by calculated gross energy using ADL and Klason lignin.

Sample	Measured Gross Energy	Calculated Gross Energy	
		ADL	Klason lignin
	kcal/kg	%	
Alfalfa	4493	80.8	97.1
Alfalfa	4371	79.1	93.1
Kura clover	4460	76.2	87.6
Annual Medic	4308	81.3	93.7
Corn silage	4392	77.2	96.9
Corn silage	4497	78.4	93.7
Orchardgrass	4485	70.1	88.1
Bromegrass	4323	74.8	96.5
Switchgrass	4377	66.1	87.8
Oat straw	4182	73.8	96.7

Revising the Acetyl Bromide Assay to Optimize Lignin Determinations in Forage Plants

R.D. Hatfield, K. Brei and J.H. Grabber

Introduction

The acetyl bromide method was originally developed to provide a rapid, yet sensitive, spectrophotometric method to determine lignin concentrations in small wood samples (Johnson et al.). It has since been modified a number of times mostly to adapt the procedure to herbaceous plants. Most recent modifications have utilized perchloric acid as a catalyst to aid in the total solubilization of plant cell walls (Iiyama and Wallis). We were interested in using this method for small forage samples and lignin fractions which are not amenable to forming insoluble residues (i.e., Lignin-carbohydrate complexes, etc.). However, difficulties which arose during routine sample analysis prompted us to re-evaluate the standard procedure to improve the reliability of lignin determinations.

Materials and Methods

For all experiments, the acetyl bromide reagent was 25% (v/v) acetyl bromide in glacial acetic acid. All samples were dried overnight at 50 °C before weighing into glass culture tubes (16 x 150 mm) fitted with Teflon lined screw caps. Weighed samples were placed in a vacuum desiccator over P_2O_5 with the caps off for at least 18h before analyzing. The standard procedure involved removing the samples from the desiccator, adding 2.5 mL of acetyl bromide reagent (prepared fresh), capping immediately, and heating at either 50, 60 or 70 °C. Some samples also contained 100 µL of perchloric acid. Heating times varied from 15 minutes to 4h depending upon the nature of the experiment. After heating, the samples were quantitatively transferred, with the aid of acetic acid, to 50 mL volumetric flasks that contained 10 mL of 2 M NaOH and 12 mL of acetic acid. Hydroxylamine (350 µL of 0.5 M) was added to each flask and samples were diluted to 50 mL with acetic acid. Absorption spectra (250 to 350 nm) were determined for each sample and used to determine the absorption maxima at 280 nm. Samples included corn rind walls, alfalfa stem walls, corn cell culture walls (lignified to varying degrees), and polysaccharides (polygalacturonan, arabinoxylan, and cellulose). All experiments were run in triplicate with duplicates for each treatment.

Results and Discussion

Initially, several parameters were tested to determine their impact upon the lignin values obtained from standard samples (corn rind and alfalfa stem cell walls). These experimental parameters included concentration of acetyl bromide, heating time, heating temperature, perchloric acid, and use of hydroxylamine. Of these parameters, acetyl bromide concentration appeared to have the least impact as long as the concentration was maintained at 15 to 30% (v/v). It was also determined that addition of hydroxylamine, as described in the original method, gave the most consistent results (i.e., removal of polybromide anion that forms during the reaction). As expected, decreasing the reaction temperature decreased the rate of reaction that could be compensated for by increasing the reaction time.

The impact of varying the reaction temperature is shown in Figure 1. Samples were heated at 50 °C for 2 h, 60 °C for 1 h and 70 °C for 0.5 h with or without the addition of perchloric acid. Theoretically the increased reaction time should compensate for the decrease in reaction temperature. This would appear to be the case for samples without the inclusion of perchloric acid (Fig. 1 dashed lines). The inclusion of perchloric acid resulted in significantly higher absorption values as the temperature increased (Fig. 1 solid lines). This could be interpreted to mean that increased temperature and the addition of perchloric acid are required to solubilize all of the wall bound lignin. Alternatively the increase

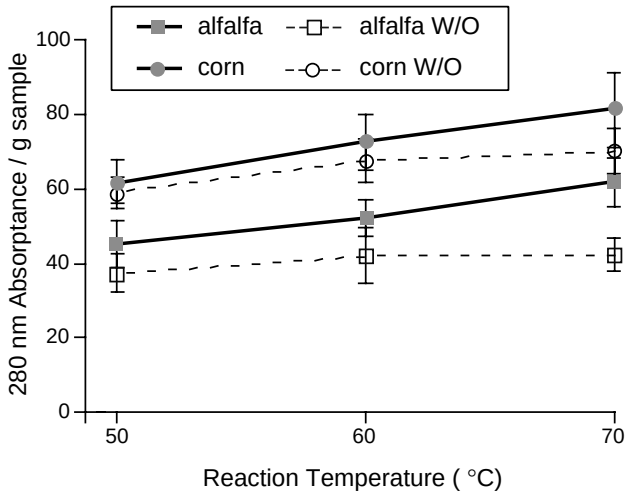

Figure 1. Changes in the total absorbance at 280 nm after treatment of cell walls with acetyl bromide reagent for 2 h at 50 °C, 1 h at 60 °C and 0.5 h at 70 °C. Solid lines represent samples with perchloric acid added and dashed lines were without perchloric acid.

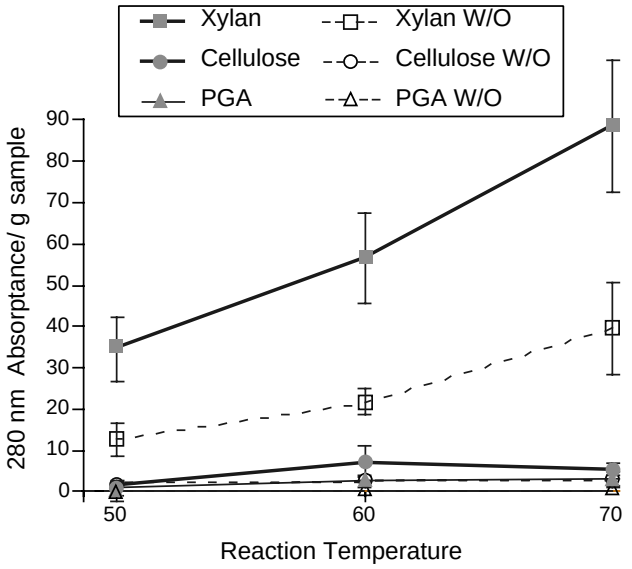

Figure 2. Reaction of wall polysaccharides with acetyl bromide at increasing temperatures. Solid lines represent samples with perchloric acid added and dashed lines were without perchloric acid. Heating times were 2 h at 50 °C, 1 h at 60 °C and 0.5 h at 70 °C.

in absorbance could be due to increased polysaccharide degradation.

Treatment of selected structural polysaccharides with acetyl bromide, following the same regime as above, revealed that xylans were particularly susceptible to degradation in the acetyl bromide reagent (Fig 2). Increased temperature accelerated this degradation and was further enhanced by the addition of perchloric acid. Although the absorption spectra for degraded xylans were different from lignin, there was a strong absorbance at 280 nm, the absorption maximum used for lignin. It is likely that a good deal of this increased absorbance at higher temperatures, and especially in the presence of perchloric acid, was due to xylan degradation. Dehydrogenation polymers of coniferyl alcohol (DHP, artificial lignins) formed in the absence of cell wall polysaccharides did not show the same temperature dependent reaction. All temperatures gave the same absorbance values (Fig. 3). There was only a slight increase when perchloric acid was added to the reaction. These findings indicate that higher absorbance values, especially with added perchloric acid, may be due to xylan degradation.

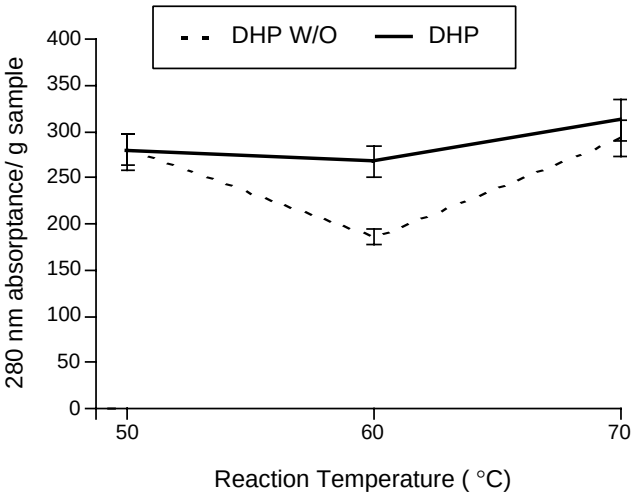

Figure 3. Comparison of DHP lignins treated with acetyl bromide reagent (heating times as in Fig. 1). Solid lines = perchloric acid added. Dashed lines = without perchloric acid.

Conclusions

The acetyl bromide assay for lignin is a convenient procedure for small samples which may not be suitable for Klason or acid detergent lignin methods. However, xylan degradation should be considered as a possible interference that would overestimate total lignin concentrations. This can be minimized by using a lower temperature such as 50 °C even though the reaction time is increased to 2 to 4 hours depending on the difficulty in wall solubilization.

Impact

Improved methods for determining lignin concentrations, especially on small samples, allow a better understanding of lignin's influence on wall digestibility. Detailed in information concerning the interactions of wall components leads to development of genetic approaches (traditional and molecular) to improve forage cell wall digestibility.

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The "DFRC" Method: a New Method for Structural Characterization of Lignins

F. Lu and J. Ralph

Introduction

During recent work it became clear that we could develop a new lignin characterization method. We had developed tandem reactions which were capable of breaking aryl ether bonds in lignins. This allows us now to provide a simplified alternative to the currently popular thioacidolysis method, one of the most important lignin structural tools.

The new method, based on derivatization and plant cell wall solubilization reactions via acetyl bromide followed by reductive ether cleavage, has been coined the "DFRC" Method for **D**erivatization **F**ollowed by **R**eductive **C**leavage (and reflecting the **D**airy **F**orage **R**esearch **C**enter where it was developed). The DFRC method is somewhat easier, more selective and significantly less malodorous than thioacidolysis.

The "DFRC" Method

Figure 1 shows the chemical basis of the method. In the first step, dissolution of the cell walls in an acetyl bromide/acetic acid solution provides soluble components allowing unrestricted access of the required sites to bromination and the following reductant and allowing rapid reactions without resorting to elevated temperatures. At the same time, the lignins are cleanly derivatized and some bond cleavage occurs. Thus α–ethers are selectively cleaved, all free OH's (phenolic and aliphatic) are acetylated, and benzylic positions become brominated. β–Ether units are not cleaved. These reactions on β–ether moieties are extraordinarily clean as long as conditions are not too harsh; some of the conditions recommended for the acetyl bromide method for lignin quantitation are too stringent and produce side reactions such as ring-acylation. In particular, we avoid the higher reaction temperatures (50 °C is sufficient, near 70 °C ring acylation becomes significant) and never use the perchloric acid advocated in recent versions of the acetyl bromide method. The acetyl bromide and solvents are then simply removed under vacuum or in an air stream — there is no need for product work-up.

The second step is the key to ether cleavage. A reducing source, zinc in acetic acid, rapidly cleaves the β–bromo ethers **1** that are produced in the first step. Again, this reaction is very high-yielding and clean. Tiny amounts of side-products from model compounds are detectable by GC-MS but are at such a low level that we need to purify our starting models to extraordinary lengths to detect real side products from those arising from trace impurities in our model compounds. At this point, all β–ethers and non-cyclic α–aryl ethers in lignin have been cleaved. Methoxyl groups remain intact.

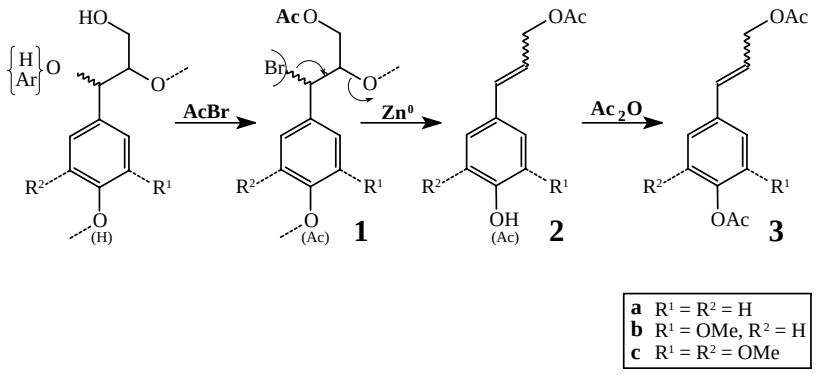

Figure 1. The reaction basis of the DFRC method. Treatment with AcBr acetylates alcohols and phenols, cleaves α-ethers, and brominates α-positions, at the same time solubilizing the whole cell wall. Two-electron reduction cleaves β-ethers. Acetylation produces the final hydroxycinnamyl acetates 3 for GC analysis.

Finally, in order to produce the simplest sample to analyze and to give quantitative data analogous to that of thioacidolysis, acetylation of phenolic hydroxyls reduces the number of distinct compounds to be quantitated. Regrettably, there are still two isomers to quantitate. In thioacidolysis, the *threo*– and *erythro*–trithioethyl

compounds result in approximately equal amounts. Here we obtain *trans*- and *cis*-hydroxycinnamyl acetates **2**, with the *trans*-isomer predominating (> 90%). When more extensive data are at hand, it might be possible to quantitate only the *trans*-isomer and calculate the total contribution from a known fixed ratio, but the *trans:cis* ratio is subtly dependent on exact reaction conditions. For crude analyses, the *cis*-isomer can simply be ignored. Attempts at removing the isomer complication, e.g. via hydrogenation, provided more complications and have now been abandoned. For now, each isomer's GC peak is largely free from contamination by other products and is readily quantitated. Figure 2 shows the results using various *p*-hydroxyphenyl/guaiacyl/syringyl model compounds; Figure 3 shows DFRC products from four lignin samples. To illustrate the cleanliness even when whole cell wall material is used, Figure 4 shows the resultant products from pine (a softwood) and bass (a hardwood).

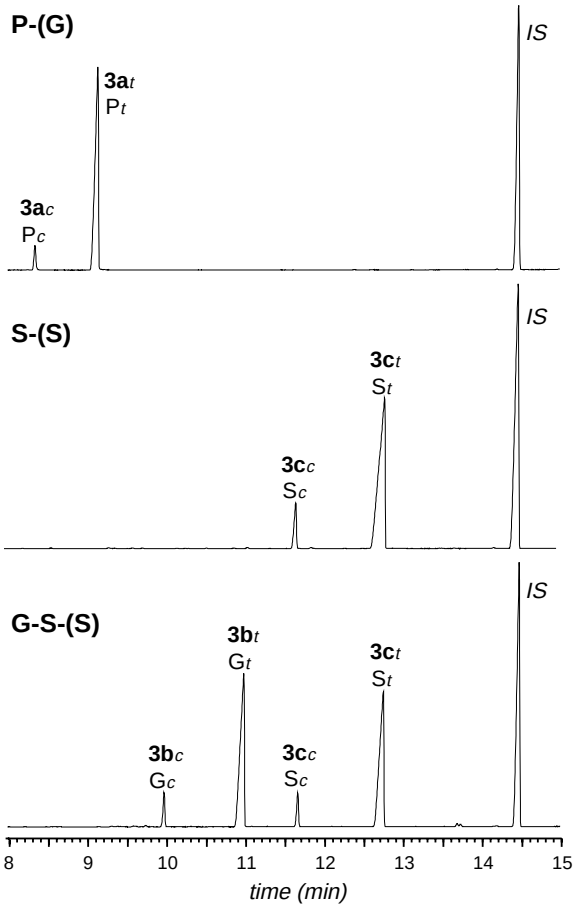

*Figure 2. GCs of DFRC products from three model compounds showing the cleanliness of the products following the three reaction steps. P, G, and S represent p-hydroxyphenyl, guaiacyl, and sinapyl units **3a-c** (Fig. 1). The final unit (in brackets) has no sidechain and is not present as such in real lignins; products from this unit appear early in the chromatogram (not shown). t = trans, c = cis .*

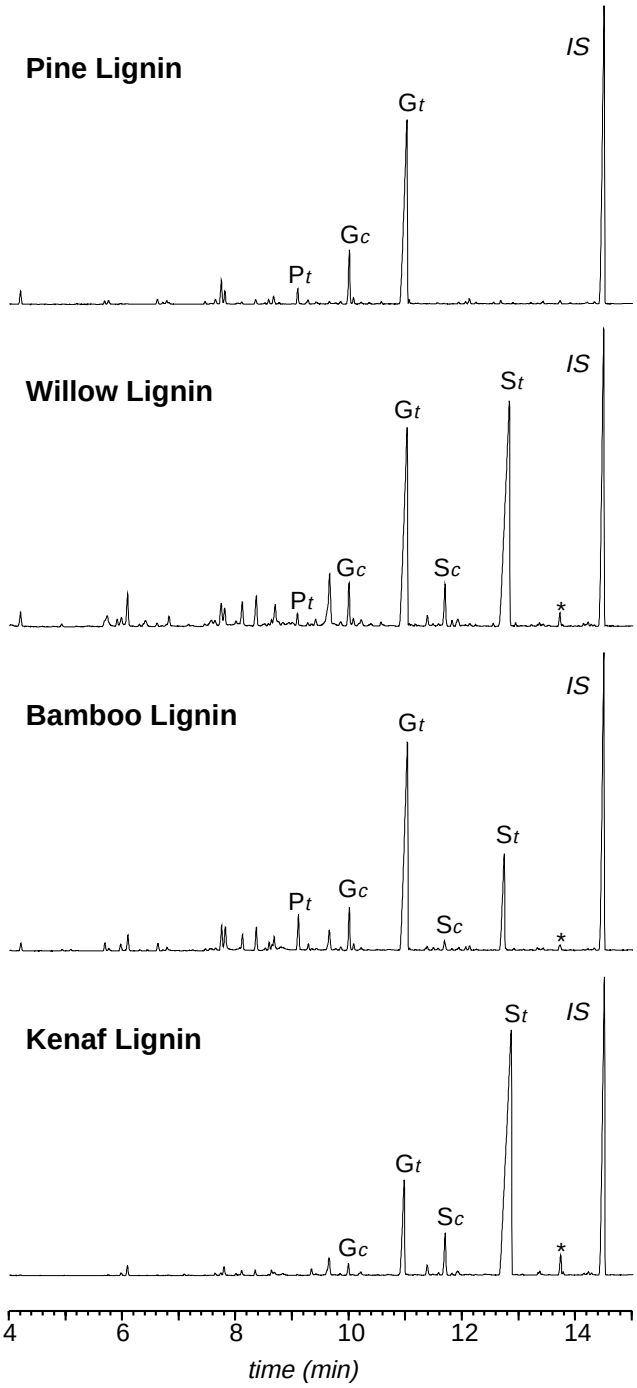

*Figure 3. GCs of DFRC products from isolated milled lignins showing the readily quantifiable P, G, and S products **3a-c** (Fig. 1). The asterisked (*) peak is from 5-hydroxyconiferyl units, detected in every S/G lignin with the DFRC method.*

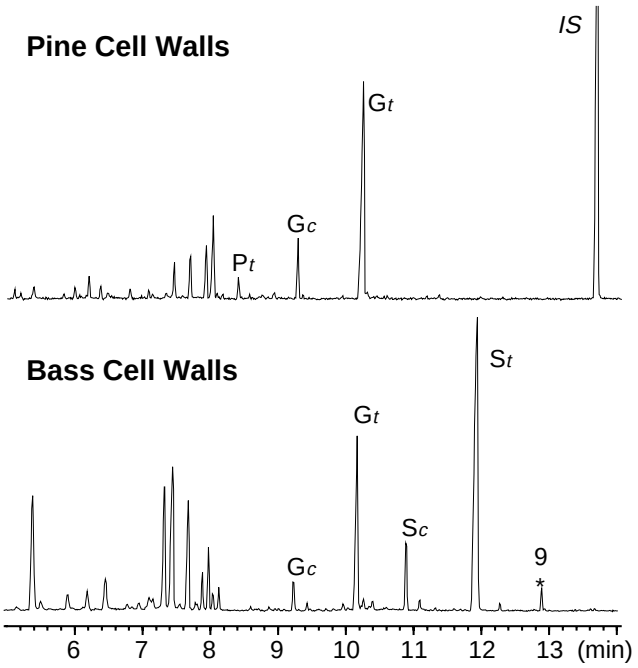

*Figure 4. Mass spec. total ion chromatograms of DFRC products from whole cell wall samples, pine (softwood) and bass (hardwood). The normal P, G and S peaks **3a-c** (Fig. 1) are shown as well as the 5-hydroxy-product. Early peaks in the chromatogram are largely polysaccharide-derived.*

Advantages of the DFRC method. In our totally biased opinions, the following advantages are seen in this method.

1. Reactions are more selective and more quantitative. As noted above, the AcBr and the Zn0 reactions are extremely clean. Reactions of model compounds through the entire process produces ~95% yields of the targeted products. Thioacidolysis yields are typically 75%. Thioacidolysis produces some chain-shortened products (aryl ethyl derivatives rather than aryl propyl) from side reactions.
2. The reactions are simpler and occur under milder conditions. Solvolytic reactions at 100+ °C under acidic conditions are not typically used in organic syntheses because they are more difficult to predict and control. The reactions described here are carried out at room temperature to 50 °C. They are sufficiently high yielding and clean to be synthetically as well as analytically useful.
3. Analyses are less sensitive to experimental conditions. Any optimization is recommended in each lab before beginning analytical determinations by thioacidolysis. Indeed in our own lab we frequently found dimeric β–ethers indicating that we are not efficiently cleaving all β–ethers. It appears that an optimization is also required when reagents are changed. The DFRC method is far less sensitive to sample and operating conditions. Although it is envisaged that some sample types may prove less amenable to AcBr reactions, the general procedure developed can be applied to a wide variety of sample types without requiring further optimization.
4. The DFRC products are stable for long periods (unlike thioacidolysis products). GC analysis can be performed and/or repeated at leisure.
5. Malodorous chemicals are not required. No discussion of thioacidolysis is complete without mention of the foul stench of the required reagent (ethane thiol). Although this can be minimized by careful work, utilizing bleach for trapping, etc., building staff are generally not supportive of thioacidolysis experiments. In some labs, it is simply not possible to run thioacidolysis because of restrictions on odor.
6. Esters are not cleaved. In thioacidolysis, esters are partially cleaved and this causes problems with grasses (for example) where *p*–coumarate esters (at the lignin γ–position) can be quite prevalent. γ–Esters remain completely intact after DFRC reactions and this can be used to tremendous advantage in identifying and characterizing esterified lignin components.
7. The scheme is amenable to targeting products and to a rather wide variety of future applications. Many are now under active investigation and will hopefully be reported in the future.
8. A lignin determination can be made at the same time as the DFRC method; the first AcBr step is similar to AcBr methods developed for lignin determination. We are currently working on integrating these methods.

Additionally the hydroxycinnamyl acetates are more diagnostic of their lignin origin than the trithioethyl derivatives or the fully saturated aryl propanes that result from the two thioacidolysis methods. For that reason, we sought to establish methods based on the hydroxycinnamyl acetates rather than aryl propanes or various unsaturated analogues that could also be targeted via modified conditions (particularly in the reduction step).

Disadvantages over thioacidolysis. The main drawbacks to the method at this point stem from its immaturity. We don't yet know what happens to a number of structural units that have already been investigated by thioacidolysis, although this number is rapidly declining. Many of these are the focus of current work.

Experimental protocol.
Acetyl Bromide Stock Solution
AcBr:acetic acid 8:92 (by volume) for model compounds and isolated lignins.
AcBr:acetic acid 20:80 (by volume) for cell wall samples.

Acetyl bromide derivatization and wall solubilization reactions. To a 10 ml round bottom flask containing about 10 mg of lignin, lignin model compound, or 20 mg cell wall sample is added 2.5 ml of AcBr stock solution. [Smaller amounts are possible for this analysis]. The mixture is gently stirred at room temperature overnight (models, lignins) or 50 °C for 3 hours (cell wall samples).

Reductive cleavage reactions. After removal of solvents by rotary evaporation at 50 °C (we think evaporation in an air stream is OK but this is still being tested), the residue is dissolved in 2.5 ml of dioxane/acetic acid/water (5:4:1, v/v/v). To the solution is added ~50 mg of zinc dust with good stirring. The mixture is stirred for 30 minutes. After addition of internal standard (tetracosane in methylene chloride, 3-5 mg for models, 300 µg for lignins, 200 µg for CW samples), the mixture is poured into methylene chloride (10 ml) and washed once with saturated ammonium chloride solution. The water phase is extracted twice with methylene chloride. The combined methylene chloride fractions are dried over anhydrous magnesium sulfate, filtered, and the solvent removed under reduced pressure. The residue is acetylated (pyridine/acetic anhydride) and the product utilized directly for NMR, GC, or GC-MS analysis.

GC quantitation. The residue from above is dissolved in methylene chloride: a) about 1.5 ml for lignin models, b) 200 µl for lignins, c) 100 µl for CW samples. A sample, 1.5 µl, is injected into a GC (Hewlett Packard 5980). [Column 0.20 mm x 30 m SPB-5 (Supelco); He carrier gas, 1 ml/min; injector 220 °C, initial column temperature 160 °C, ramped at 10 °C/min to 300 °C, hold 5 min; FID detector, 300 °C]. Response factors, determined from independently synthesized products, relative to the tetracosane internal standard and relative retention times are given in Table 1.

Conclusions
Although most of the examples given here are for woods, the method works equally well for grasses and legumes that form the basis of our core research at the Center. Applications of this method will help enormously with the characterization of forage wall components and structure. Further future extensions to this basic methodology stand poised to provide an arsenal of useful tools with which to tackle mysteries of cell wall structure and provide insights into the limitations to digestibility of its polysaccharides.

Table 1. Relative Retention Times (RRT) and Response Factors (RF) relative to tetracosane internal standard, for the DFRC-released lignin monomers.

	cis-**3a**	*trans*-**3a**	*cis*-**3b**	*trans*-**3b**	*cis*-**3c**	*trans*-**3c**
RF	1.76	1.76	1.84	1.84	2.20	2.20
RRT	0.58	0.63	0.69	0.76	0.81	0.88

New Discoveries Relating to Diferulates

J. Ralph, J.H. Grabber, R.D. Hatfield and G. Wende

Introduction

A couple of years ago, we reported on the discovery of a whole class of dehydrodimers of ferulic acid in grasses (Ralph et al. 1994). Prior to that time, only one dehydrodimer (the 5–5-coupled dimer) was recognized and diferulates as a whole were under-quantitated by factors of up to 20. Since this time, collaborations with other labs have led to discoveries of diferulates in a wide variety of plant materials. Some of the recent findings are quite novel; others suggest enormous potential for utilization of ferulate dimerization in producing new food products. The importance of diferulates in cross-linking the plant cell wall is becoming recognized.

Implication of diferulates in hypocotyl growth-cessation. Researchers in Spain (Sánchez et al. 1996) have been studying the mechanism by which pine hypocotyl growth cessation occurs once the top of a germinating seedling encounters light. Such a light-response might be expected to be photochemical in nature but the plant halts extension of the cell walls (that would continue to occur in the dark) by infusing the cells with peroxidases and effecting ferulate dimerization. Cross-linking of the walls by diferulate bridges effectively prevents further expansion of these cells and allows the plant to move on to the next phase of its growth — producing shoots and leaves. What is intriguing in this case is that a range of diferulates was found (when pine cell wall development has not normally been considered to involve ferulates) and that none of the 5–5-coupled diferulates could be detected. Such an absence is more in line with the free-solution coupling of model ferulates which produces other dimers but not the 5–5-coupled isomer. Presumably this mechanism for growth-cessation could have been overlooked if researchers sought only the 5–5-coupled dimer as has been done for the past 20 years. The only other study of this type used rice coleoptiles. The 5–5-coupled dimer was observed to accompany growth cessation there. Recent studies in this lab show that the production of the full range of dimers (not just the 5–5) accompanies the growth cessation.

Diferulates in sugar beets. Sugar beets provide the food industry with many useful products in addition to the sugar that is produced in some countries. The beet pulp is a valuable source of pectins and has many uses in the food industry.

Collaborations with two groups in France (Micard et al. 1996) and Holland (Oosterveld et al. 1996) have revealed considerable amounts of all of the diferulates in sugar beet pulp. What is particularly important in this case is that the propensity for further ferulates in the pulp to dimerize (effecting polysaccharide cross-linking again) can be utilized to change the pulp properties. Thus a simple treatment of the pulp with peroxidase and hydrogen peroxide produces more dimers (more cross-linking) and consequently alters the pulp viscosity. Such a product then becomes amenable to further uses in the food industry, opening up further markets for its utilization.

Diferulates in Chinese water chestnuts, and a new method for determining diferulates. A lab in the UK (Waldron et al. 1996) has discovered that there are substantial levels of the range of diferulates in water chestnuts and is now attributing the crunchy texture, even after cooking, to these intriguing little molecules. Another collaboration (Parr et al. 1996) has resulted in an alternate HPLC method for quantitation of diferulates with compound identification via diode array spectroscopic detection; our established method utilizes GC for compound separation and quantitation, GC-MS for product identification. The availability of an alternate method opens up the analysis of these important wall components to more laboratories.

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Diferulate Cross-Links Limit the Degradation Rate of Nonlignified Maize Walls by Fungal Hydrolases

J.H. Grabber, R.D. Hatfield and J. Ralph

Introduction

Ferulic acid is esterified to the C5-hydroxyl of a-L-arabinose moieties of grass xylans. Xylans are cross-linked by oxidative coupling of ferulate monomers into dehydrodimers. The substitution and cross-linking of xylans by ferulates is thought to limit the enzymatic degradation of grass walls, but unambiguous evidence for such a role is lacking. We specifically manipulated ferulate substitution and diferulate cross-linking in nonlignified walls to elucidate how ferulates restrict fiber degradation by fungal hydrolases.

Methods

Maize cell suspensions (*Zea mays* cv. Black Mexican) were grown with 0 to 50 µM 2-aminoindan-2-phosphonic acid (AIP) to manipulate the deposition of ferulate esters into nonlignified walls. In a separate study, nonlignified walls from cell suspensions grown with 0 or 40 µM AIP were incubated with mercaptoethanol to inhibit diferulate formation or with dilute hydrogen peroxide to stimulate diferulate formation by wall-bound peroxidases. Cell walls were analyzed for neutral sugars, uronic acids, hydroxycinnamic acids, and lignin. Cell walls were degraded with hydrolases from *Trichoderma reesei* (Celluclast, NOVO) and *Aspergillus niger* (Viscozyme L, NOVO). Periodically during enzymatic hydrolysis, wall residues were pelleted by centrifugation and an aliquot of the supernatant was analyzed for total carbohydrate, uronic acids, and for neutral sugars after hydrolysis with 2 N trifluroacetic acid. The kinetics of sugar release were described using a first order model.

Results and Discussion

Cell walls from maize cell suspensions contained 197 mg g^{-1} of arabinose, 180 mg g^{-1} of xylose, 75 mg g^{-1} of galactose, 320 mg g^{-1} of glucose, 110 mg g^{-1} of uronic acids, 17 mg g^{-1} of ferulate esters, trace amounts of ferulate ethers, 0.5 mg g^{-1} of *p*-coumaric acid, and 3 mg g^{-1} of guaiacyl lignin. Overall, the chemical composition of cell walls was representative of nonlignified primary walls of grasses. Ferulate ester deposition into cell walls was reduced up to 75% by growing cell suspensions in the presence of AIP, a specific inhibitor of phenylalanine ammonia lyase. Hydrogen peroxide treatment of walls with normal or low feruloylation increased the proportion of diferulates to total ferulates from *ca* 18% to 44% (Table 1). Other wall components were not modified significantly by AIP and H_2O_2 treatments.

Concurrent reductions in ferulate substitution and cross-linking caused by AIP treatment increased the release of carbohydrate from walls by fungal enzymes. When walls with normal or low feruloylation were treated with hydrogen peroxide, peroxidase-mediated coupling of ferulate monomers into dehydrodimers reduced carbohydrate release by 122 mg g^{-1} after 3 h and by 48 mg g^{-1} after 54 h of enzymatic hydrolysis (Table 1). These results provide compelling evidence that the enzymatic hydrolysis of walls was controlled by diferulate cross-linking and not by ferulate substitution of xylans. Averaged over all treatments, release rate was greatest for galactose and uronic acid (*ca* 0.265 h^{-1}), intermediate for arabinose and glucose (*ca* 0.160 h^{-1}), and lowest for xylose (0.093 h^{-1}). Hydrogen peroxide treatment reduced the rate of sugar release by an average of 42% (Table 2), indicating that diferulate cross-linking of xylans restricted the rate at which all polysaccharides were released from walls. Averaged over all treatments, the extent of sugar release from walls was slightly greater for glucose and galactose (*ca* 0.884) than arabinose, xylose and uronic acids (*ca* 0.858). Alteration of ferulate substitution and cross-linking generally had little effect on the extent of sugar release from walls. However, hydrogen peroxide treatment of walls with normal feruloylation reduced arabinose release by 8% and xylose release by 21%, suggesting that the degradation of xylans was reduced by high levels of diferulate cross-linking.

Conclusions

Diferulate cross-linking of arabinoxylans impedes the release of all polysaccharides from nonlignified walls by fungal enzymes. Except for xylans, the extent of polysaccharide degradation was not affected by diferulate cross-linking. Simple substitution of xylans with ferulates did not affect wall hydrolysis.

Impact Statement

Research with this cell-wall model system provides a unique means of elucidating factors which limit efficient utilization of cell walls for nutritional and industrial purposes. Ultimately, these studies should allow rational approaches to maximizing plant utilization and farm sustainability while minimizing adverse impacts on the environment.

Table 1. Ferulate composition and fungal hydrolase degradability of nonlignified maize walls (n = 2). Feruloylation of cell walls was manipulated by growing maize cell suspensions with and without AIP, a specific inhibitor of phenylalanine ammonia lyase. Peroxidase-mediated coupling of ferulate monomers into dimers was limited by isolating and incubating cell walls with mercapoethanol or stimulated by incubating cell walls with hydrogen peroxide.

		Ferulate esters			Total carbohydrate released	
AIP	H_2O_2	monomers	dimers	total	3 h	54 h
µM	mmol	- - - - - - - - - mg g^{-1} cell wall - - - - - - - - -			-mg g^{-1} wall carbohydrate-	
Normal feruloylation						
0	0	14.53	2.62	17.15	357	856
0	0.4	8.96	6.65	15.61	243	794
Low feruloylation						
40	0	3.75	1.31	5.06	460	898
40	0.4	2.27	2.25	4.52	329	865
Analysis of Variance						
AIP		*	*	*	*	*
H_2O_2		*	*	NS	*	*
AIP X H_2O_2		*	*	NS	NS	NS

* = Significant at the 0.05 level of probability.
NS = Not significant.

Table 2. Rate of sugar release during fungal hydrolase degradation of nonlignified maize walls as affected by hydrogen peroxide treatment of nonlignified walls (averaged over AIP treatments).

H_20_2	Diferulate	Total ferulate	Arabinose	Xylose	Galactose	Glucose	Uronic acids
mmol	- - mg g^{-1} of cell wall - -		- - - - - - - - - - -Rate constant (h^{-1}, LSD† = 0.025) - - - - - - - - -				
0	1.97	11.11	0.186	0.127	0.323	0.229	0.337
0.4	4.45	10.07	0.099	0.059	0.175	0.132	0.230

†LSD to compare means within columns (P = 0.05).

Preparation of Three Coniferaldehyde Dimers and a Trimer

F.H. Ludley and J. Ralph

Introduction

Structural elucidation of lignin by NMR relies strongly on comparison with synthetic model compounds. As described in the previous article, coniferaldehyde-containing lignins are of current interest because of the discovery of naturally CAD-deficient mutants and the current interest in antisense technology for down-regulating CAD activity in various plants. The preparation of three coniferaldehyde dimers and one trimer is described.

Method

Coniferaldehyde **1** was dissolved in acetone, and silver(I) oxide was added to the solution. The resulting suspension was stirred at room temperature for 2.5 h. The solids were filtered off, washed thoroughly with acetone and the combined solvent fractions evaporated. The resulting orange oil was separated into its components by SiO_2 thick layer chromatography. To achieve optimal separation, the plate was run several times in different solvent combinations with increasing polarity. The fractions are scraped off the plate and extracted with acetone. Three dimers **2**, **3** and **4** and one trimer **5** were isolated and have been characterized.

Conclusion and Discussion

A method has been described to prepare coniferaldehyde dimers **2**, **3** and **4** as well as the trimer **5** as a mixture, by radical reactions. It is based on radical coupling reactions effected by silver(I) oxide that have been previously utilized to prepare more conventional lignin dimers (Research Summaries 1992). NMR-spectroscopic characterization of these compounds has been completed and data are deposited in the "NMR Database of Model Compounds for Lignin and Related Cell Wall Components," available over the Internet from our sites at http://www.dfrc.wisc.edu or http://www-cwg.dfrc.wisc.edu. These compounds and their data will be used to more fully elucidate how coniferaldehyde is incorporated into plant lignins. The presence or absence of these model compounds in the polymers provides an insight into biosynthetic processes in plants and will facilitate methods for producing plants with improved digestibility.

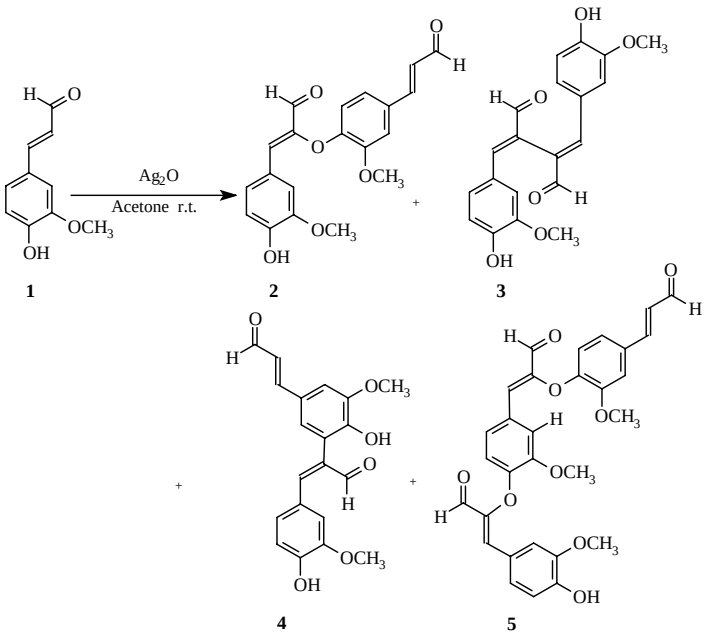

*Figure 1. Synthetic scheme used to prepare coniferaldehyde dimers **2-4** and trimer **5**.*

Synthesis of ^{13}C-labeled Coniferaldehyde and Its Incorporation into Synthetic Lignins

F.H. Ludley and J. Ralph

Introduction

Lignins are formed in plants by peroxidase/H_2O_2-induced polymerization of *p*-coumaryl, coniferyl and sinapyl alcohols. In plants where CAD (cinnamyl alcohol dehydrogenase) activity is low, *p*-hydroxycinnamaldehydes presumably build up and could become a major component of the lignin produced. Cinnamaldehyde-containing lignins have been shown to severely inhibit fiber degradation in forages. To determine lignification pathways and to explain the degradation behavior, it is necessary to determine the structure of CAD deficient lignins. Comparison of synthetic lignins (dehydrogenation polymers, DHPs) with plant lignins should identify structural features and provide an understanding of the differences in forage digestibility. A synthetic pathway to DHPs is presented using [γ-^{13}C]coniferaldehyde **8**.

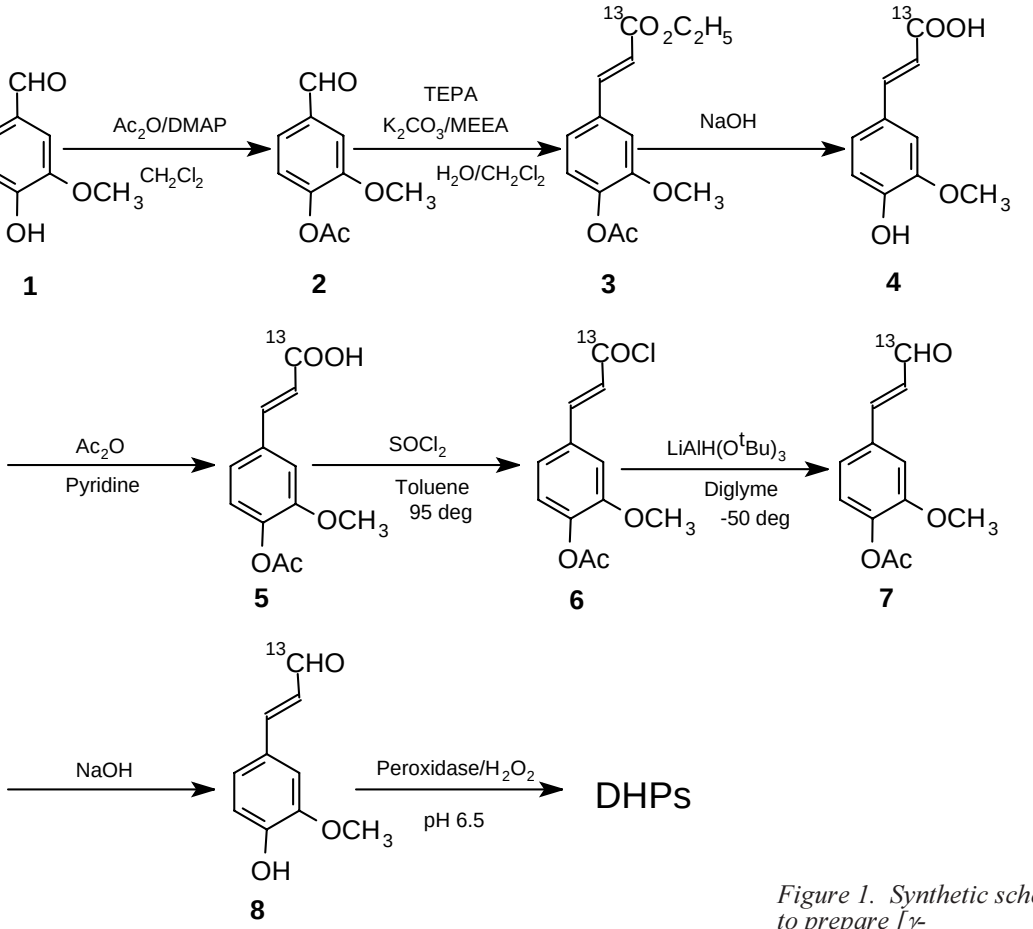

*Figure 1. Synthetic scheme used to prepare [γ-^{13}C]coniferaldehyde **8** and synthetic lignins (DHPs).*

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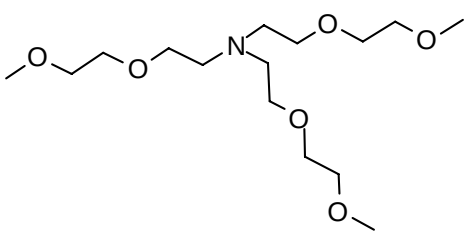
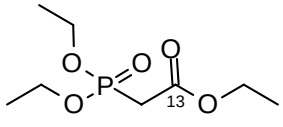


TEPA:



Method

Reaction of vanillin **1** with acetic anhydride/4-N,N-dimethylaminopyridine (DMAP) in methylene chloride gives 4-O-acetylvanillin **2**. The ^{13}C-label is introduced via a Wittig-Horner-Emmons reaction of **2** with [1-^{13}C]triethyl-phosphonoacetate in a two-phase reaction system of water/methylenechloride using potassium carbonate as base and tris[(methoxye-thoxy)ethyl]amine as a phase-transfer-catalyst. Saponification of the ester **3** under strongly alkaline conditions yields [γ-^{13}C]ferulic acid **4** which has to be reacetylated to give [γ-^{13}C]4-O-acetylferulic acid **5**. Reaction of **5** with thionyl chloride in toluene at 95° C yields the acid chloride **6** which is not isolated but reduced immediately to [γ-^{13}C]4-O-acetylconiferaldehyde **7** using lithium-tri-*t*-butoxyaluminum-hydride in diglyme at -50° C under inert conditions. Deacetylation of **7** with aqueous sodium hydroxide leads to the target molecule **8**.

Two different dehydrogenation polymers (DHPs) were prepared using [γ-^{13}C]coniferaldehyde **8**. The first was made by using 10% **8** and 90 % unlabeled coniferyl alcohol to elucidate cross reactions between the normal lignin monomer (coniferyl alcohol) and the aldehyde component. The second was made by reacting pure [γ-^{13}C]coniferaldehyde **8** under lignification conditions to elucidate homo-coupling reactions in cases where the aldehydes are in high concentration. Preparation of the DHPs was done using the "Zutropf-Method" where a solution of **8**, or **8** and coniferyl alcohol, was added dropwise to a mixture of peroxidase and H_2O_2 in a buffer solution.

Discussion and Conclusion

A method has been developed to prepare [γ-^{13}C]coniferaldehyde **8**. The pathway utilized the previously described (Research Summaries 1991) way of easily introducing the ^{13}C label by using commercially available [1-^{13}C]triethyl-phosphonoacetate in the Wittig-Horner-Emmons-reaction to produce ferulate. The aldehyde has now been used to prepare synthetic lignins (DHPs). NMR spectroscopy of these DHPs should elucidate radical coupling pathways that are accessible to coniferaldehyde and, along with the model data (see following article), provide the necessary data required to more carefully characterize lignins from CAD-deficient plants. Knowledge of which pathways and products can be selected for in plant breeding, or can be genetically altered, should ultimately result in plants that are more digestible and therefore provide greater sustainability to the farm system.

Corn Cell Wall Peroxidases: Potential Role in the Lignification Process

R.D. Hatfield, C. Neumeier, J.H. Grabber and J. Ralph

Introduction

In general, plants produce several isozymes of peroxidase. Corn cell walls contain at least 12 to 15 different isozymes with peroxidase activity. The role of these isozymes in plant development is not clear. We have investigated three major groups of peroxidases isolated and partially purified from the walls of corn suspension culture cells. Previous work suggested that all peroxidases were equally adept at forming dehydrodimers of ferulate model compounds and that there was no difference in the types of dimers formed. We have now extended this work to investigate the potential role in the formation of lignin polymers.

Materials and Methods

Peroxidases were isolated from the walls of corn suspension cultures and partially purified using DEAE anion exchange and chromatofocusing chromatography. Three major groups, differing in their isoelectric points, were used in these studies (group I pI 9-10, group II pI 7-8, and group III pI 3-5). For comparison, peroxidases were also extracted from the apoplastic space of corn stem segments using gentle centrifugation (700 Xg) and a 20 mM acetate buffer (pH 5.0 200 mM $CaCl_2$). After removing apoplastic materials, stem segments were homogenized in cold (4 °C) 50 mM acetate buffers and the walls collected after washing with cold buffer, cold acetone (-20 °C), and again with cold buffer. Walls were suspended in 200 mM $CaCl_2$ overnight to extract wall bound peroxidases. Peroxidase activity was evaluated using ethyl ferulate (Et-FA), methyl *p*-coumarate (Me-*p*CA), coniferyl alcohol (CA), and sinapyl alcohol (SA). All activity reactions (total volume 1 mL) were run in acetate or MES buffers (pH 5.0) containing the appropriate substrate (0.14 to 0.16 µmoles) with hydrogen peroxide (10 µL of 7.5 mM solution) added to initiate the reaction. Rates of reactions were determined by continuously monitoring (total of 5 min) the change in absorbance at the appropriate wavelength for each substrate (ethyl ferualte, 320 nm; methyl *p*-coumarate, 308 nm; coniferyl alcohol, 261 nm; and sinapyl alcohol, 270 nm).

Results and Discussion

Peroxidase (POD) activity extracted from corn suspension cells varied with the type of substrate and the pI group (Table 1). Generally for all POD groups, the order of substrate reactivities was Et-FA > Me-*p*CA $\geq$ CA >> SA. Only Group I POD had a reasonable activity against sinapyl alcohol; all others were 20 to 40 times less active.

A comparison of POD activity extracted from corn stem walls was different from the suspension cultures in substrate preference such that Et-FA > CA > Me-*p*CA >> SA (Table 2). We evaluated different regions (upper and lower) within stem internodes and progressively more mature internodes (Int 2 = second fully expanded internode above the soil line, Int 5 = fifth internode, Int 7 = seventh internode, least mature). For grasses, like corn, the upper portion of the internode is the most mature part and the lower less mature. Substrates Et-FA and CA showed clear trends of increased activity with maturity while Me-*p*CA and SA did not reveal consistent trends with maturity. The $CaCl_2$ extracts of stem walls after homogenization showed the same activity trends as the centrifugation method (data not shown).

We had thought that perhaps the more mature portions of the stem might produce POD enzymes having higher specificity for sinapyl alcohol since, as the stem matures, there is an increase in the sinapyl monomers incorporated into lignin. This was clearly not the case (Table 2). We tested a hypothesis proposed by Takahama et al. (1996) that hydroxycinnamic acids may aid in the incorporation of sinapyl alcohol monomers into lignin. The addition of as

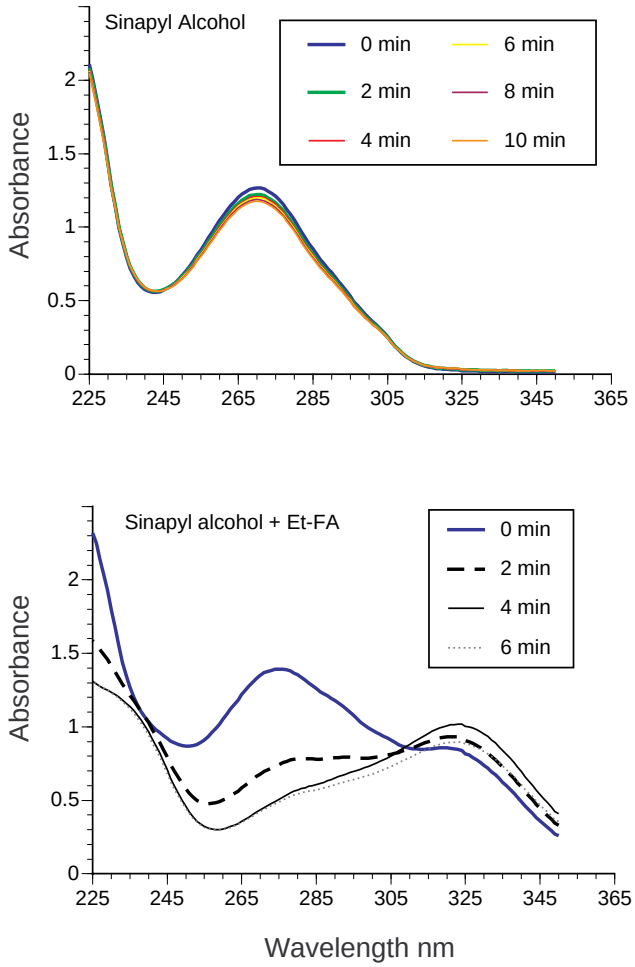


Figure 1. Spectral changes of sinapyl alcohol during coupling reactions mediated by cell wall peroxidases. A. Sinapyl alcohol with POD Group I and H_2O_2 B. Same as A except 0.045 µmoles of ethyl ferulate was included in the reaction mixture.

Table 1. Comparison of POD isozymes isolated from corn suspension culture walls. Specific acitivity towards different substrates (ethyl ferulate, Et-FA; methyl *p*-coumarate, Me-*p*CA; coniferyl alcohol, CA; and sinapyl alcohol, SA).

	Specific activity (µmoles/mg/sec)			
Isozyme Group	Et-FA	Me-*p*CA	CA	SA
Group I	12.85	8.33	5.12	1.55
Group II	2.88	0.87	1.40	0.04
Group III	3.77	2.03	2.57	0.07

little as 0.045 µmoles of either Et-FA or Me-*p*CA to a reaction mixture containing 0.15 µmoles of SA increased the rate of reaction by 30 to 50 times (Fig 1).

Conclusion

The cell walls of corn contain a wide range of POD isozymes that can vary in their substrate specificity to some extent. It would not appear, however, that there is a specific POD for the incorporation of sinapyl alcohol monomers into the latter stages of lignification. It does seem plausible that incorporation of hydroxycinnamic acids into the wall may serve a role in transferring radicals to SA allowing their incorporation into lignin.

Impact

A complete understanding of wall enzymes, such as the peroxidases, provides a clear picture of the metabolic role they play in determining the structure and function of plant cell walls. This provides a larger knowledge base from which we can develop appropriate strategies for improving the digestiblity of grasses (such as corn) without losing hardiness, pest resistance, or yield.

Reference

Takahama, U, Oniki T, and Shimokawa, H. 1996 A possible mechanism for the oxidation of sinapyl alcohol by peroxidase-dependent reactions in the apoplast: Enhancement of the oxidation by hydroxycinnamic acids and components of the apoplast.

Table 2. Comparison of POD acitivities isolated from different corn stem internodes and internode regions. Different substrates included ethyl ferulate, Et-FA; methyl *p*-coumarate, Me-*p*CA; coniferyl alcohol, CA; and sinapyl alcohol, SA.

Corn Stem	Activity (µmoles/min)			
internode	ET-FA	Me-*p*CA	CA	SA
7U	45.89	6.18	29.00	1.30
7L	37.06	4.67	27.48	2.44
5U	71.76	8.96	44.53	1.46
5L	57.75	6.88	36.86	1.22
2U	77.84	12.94	49.02	1.87
2L	79.32	9.21	47.24	1.38

Dibenzodioxocins in Forage Plant Lignins

J. Ralph

Introduction

Dibenzodioxocins were discovered in plant lignins by a Finnish group. They are exciting firstly because they represent a new structure in lignin that had not been discovered in many decades of research. Furthermore, they explain why analytical methods to quantify non-cyclic α-aryl ether units in lignins claim significant amounts (6-9%) of such structures when diagnostic NMR experiments have shown there to be typically less than 0.3%! As it turns out, the wet chemical analytical methods drew contributions for such cyclic structures. The importance lies in the fact that non-cyclic α-aryl ethers have been ascribed as important branching points in the lignin polymer formed by non-radical reactions; dibenzodioxocins do not fill that role in the same manner.

Utilizing NMR it is possible to identify dibenzodioxocins in a wide variety of lignified materials. They can be difficult to detect in un-acetylated samples due to the smearing of these peaks caused by subtle shifts in both dimensions, but are readily detected in acetylated samples.

The most important aspect impacting our understanding of forage cell walls is the finding that 5–5-coupled diferulates will also form these structures. As noted in an earlier article, diferulates are enormously important in cross-linking cell walls in grasses and reduce the digestibility of those cell walls.

Experimental

A synthetic lignin was prepared incorporating 5–5-coupled diferulate as described in last year's Research Summaries (http://www.dfrc.wisc.edu/contents.html). NMR conditions for the HSQC spectrum: 750 MHz; Bruker pulse program "invietgs" — a 2D ^{1}H-^{13}C correlation via double INEPT transfer using trim pulses, phase sensitive using echo/antiecho TPPI, gradient selection, decoupling during the acquisition; 4K by 256 increments of 16 scans collected; SW's 11 ppm, 150 ppm giving acquisition FID resolutions of 2 and 110.5 Hz/pt. Processing used Gausian multiplication (GB 0.01, LB -0.3 Hz) in F_2 and cosine-squared bell apodization in F_1.

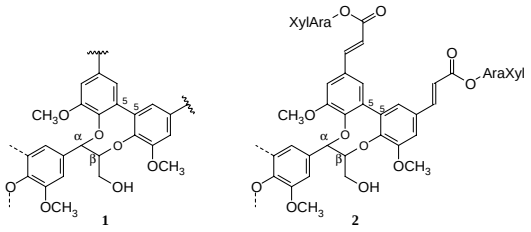


*Figure 1. Dibenzodioxocin structures from 5,5-units in normal lignin **1**, and from 5–5-coupled diferulates in grass lignins **2**.*

Results and Discussion

Utilizing the exceptional power of 750 MHz NMR with pulsed-field gradients, the diferulate dibenzodioxocins of structure 2 are readily identified in HSQC spectra (^{13}C—^{1}H correlation spectra). HMBC and HMQC-TOCSY spectra (not shown) provided further confirmatory evidence by correctly correlating other diagnostic resonances. Unfortunately, due to spectral congestion in this diagnostic area of the NMR spectra, we are currently having trouble authenticating these structures in real forage lignin samples.

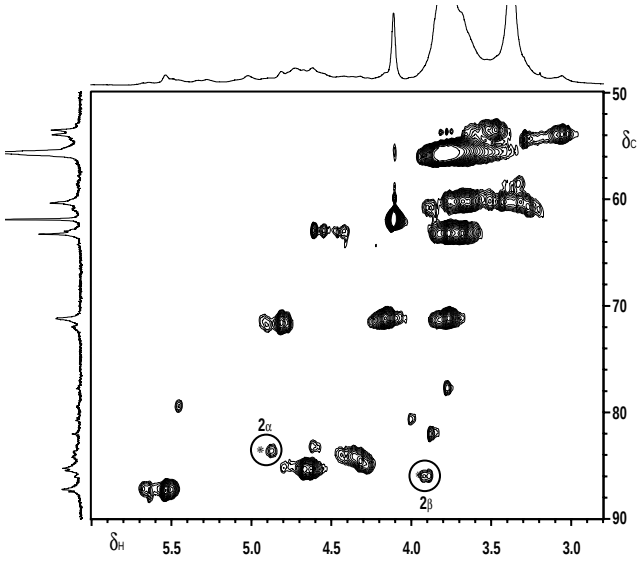


*Figure 2. Partial (sidechain region) 750 MHz gradient-selected HSQC spectrum showing clear evidence for the dibenzodioxocin structures **2**, circled. Correlations from a parent model compound of **2** are shown as *'s.*

The appearance of 5–5-coupled diferulates in these structures helps explain why their releasability is similar to that of monomeric ferulates that are incorporated into lignins. Basically, when one of the ferulate moieties radically couples to C_β of coniferyl or sinapyl alcohol during the early stages of lignification, the phenol on the second moiety becomes tied up by reacting at the α-position of the same lignin monomer. These α,β-di-ethers should be readily released by high-temperature base, or by our new "DFRC" method (see accompanying article).

Identifying these powerful cell wall cross-linking mechanisms helps in our understanding of limitations to plant polysaccharide degradibility by ruminants, and hopefully will eventually lead to rational solutions to the problem.

Chemical Composition and Degradability of Xylem and Nonxylem Walls Isolated From Alfalfa

J.H. Grabber, R.D. Hatfield, M. Panciera and P. Weinberg

Introduction

The digestibility of forage legumes declines significantly with plant maturation due to decreasing digestibility of stems and their increasing contribution to plant dry matter. Microscopic studies indicate that, as stems mature, xylem and interfascicular parenchyma form a ring of tissue that is highly resistant to digestion. Other tissues, including pith parenchyma, mesophyll, phloem, phloem fibers, collenchyma, and epidermis appear to have high degradability even in mature stems. These two types of tissues were isolated from alfalfa internodes and analyzed for cell wall composition and carbohydrate degradability as an initial step to elucidate factors limiting fiber degradability in legumes.

Methods

Xylem (xylem and interfascicular parenchyma) and nonxylem tissues (pith parenchyma, mesophyll, phloem, phloem fibers, collenchyma, and epidermis) were isolated by dissection from internode 1 (basal) to internode 6 of Vernal alfalfa plants harvested at the late bud to early flower stage of development. Crude cell walls were prepared by sequentially treating milled tissues with 50 mM NaCl, 80% ethanol, amylase, water, and acetone. Cell walls were analyzed for neutral sugars, uronic acids, hydroxycinnamic acids, and acetyl bromide lignin. Cell walls were degraded with hydrolases from *Trichoderma reesei* (Celluclast, NOVO) and *Aspergillus niger* (Viscozyme L, NOVO). After 3 and 48 h of enzymatic hydrolysis, wall residues were pelleted by centrifugation and an aliquot of the supernatant was analyzed for uronic acids and for neutral sugars after hydrolysis with 2 N trifluroacetic acid.

Results and Discussion

The cell wall content of alfalfa tissues (estimated by summing neutral sugars, uronates, and lignin) was 604 mg g^{-1} for whole internodes, 727 mg g^{-1} for xylem and 497 mg g^{-1} for nonxylem. These values slightly underestimate the cell wall content of tissues because they do not include structural proteins and small quantities of pectic sugars removed during preparation of crude cell walls. The carbohydrate fraction of all tissues was composed primarily of glucose (*ca* 60%). The proportions of other sugars differed considerably between tissue types (Table 1). On a cell wall basis, the lignin content of xylem (288 mg g^{-1}) was about two-fold greater than that of nonxylem (168 mg g^{-1}). Both tissue types contained only trace amounts of ester- and ester/ether-linked hydroxycinnamic acids.

The loss of sugars from alfalfa internodes incubated with fungal enzymes was similar to that reported for alfalfa stems degraded with rumen microorganisms (Table 1). The release of total sugars from xylem walls was extremely low, due in part to their high lignin content. Nonxylem walls, with 42% less lignin, had a 340% greater release of total sugars than xylem, indicating that the effects of lignification on wall degradability differed between tissue groups. The release of all sugars from xylem walls was low, especially for xylose and uronates (probably glucuronic acid). In nonxylem tissues, the release of most sugars was relatively high except for xylose. These results suggest that the degradability of glucuronoxylans in both xylem and nonxylem tissues is limited, possibly due to interactions with lignin. Unlike grasses, interactions between xylans and lignin in alfalfa do not appear to involve ferulic acid. Further work is needed to identify how lignins restrict the degradation of xylans and other associated polysaccharides in legumes.

Conclusions

The degradability of xylem was much lower than nonxylem walls, but in both types of walls the degradation glucuroxylans was extremely low. Elucidation of lignin-glucuronoxylan interactions is required for developing alfalfa germplasm with improved fiber degradability.

Impact Statement

Studies of cell wall composition, structure, and degradability provide basic information needed for elucidating and rectifying factors which limit fiber digestion of alfalfa. Improved fiber degradability of alfalfa would reduce both feed costs and manure output for livestock operations.

Table 1. Chemical composition and fungal hydrolase degradability of alfalfa tissues averaged across internodes.

	Rhamnose	Arabinose	Galactose	Uronates	Mannose	Glucose	Xylose	Total Sugars	Lignin
	- - - - - - - - - - - - mg g^{-1} total sugars -							mg g^{-1} dry matter	
Chemical composition									
Internode	12	32	25	106	29	611	184	460	144
Xylem	8	9	14	77	23	616	253	517	209
Nonxylem	18	59	36	150	32	593	113	413	83
Proportion of sugars released after 3 h									
Internode	0.625	0.668	0.548	0.377	0.348	0.260	0.106	0.274	
Xylem	0.272	0.421	0.404	0.043	0.208	0.154	0.048	0.127	
Nonxylem	0.840	0.708	0.677	0.770	0.478	0.430	0.280	0.499	
Proportion of sugars released after 48 h									
Internode	0.701	0.844	0.649	0.497	0.591	0.471	0.126	0.435	
Xylem	0.371	0.638	0.413	0.108	0.324	0.249	0.066	0.200	
Nonxylem	0.847	0.855	0.758	0.773	0.781	0.709	0.279	0.687	

Degradability of Cell-Wall Constituents in Corn Internodes During Stalk Development

T.A. Morrison, H.G. Jung and D.R. Buxton

Introduction

Cell-wall concentration in forage dry matter and its degradability greatly influence availability of forages to livestock that consume it. Because forage crops represent the major feed resource for cattle, improvement in dry matter digestibility and wall degradability are primary goals of many research programs. Polymerized cell-wall lignin and associated hydroxycinnamic acids [primarily ferulic acid (FA) and *p*-coumaric acid (PCA) in grass species], laid down during plant development, are thought to be the primary limiters to microbial degradation of wall polysaccharides by ruminants. The manner in which lignin and hydroxycinnamic acids limit degradability of walls is not fully understood. In some studies lignin composition and presence of hydroxycinnamic acids in the wall were more closely related to wall degradability than was lignin concentration. Cross linkage of lignin with feruloylarabinoxylan polysaccharides in the wall by FA may play a major role in explaining how lignification limits wall degradability in grasses.

During corn stalk growth, individual internodes develop sequentially at different rates. Within a growing internode the younger, undifferentiated tissues are near the intercalary meristem at the base of the internode and become progressively more developed higher up the internode. Progressive incorporation of hydroxycinnamic esters, especially FA during primary wall formation, is thought to be a key activity allowing lignin to limit wall degradability. During internode cell-wall development, polysaccharides, uronic acids, and hydroxycinnamic acids are deposited in a sequential fashion through the middle lamella, primary, and secondary walls. As lignification is initiated, beginning in the middle lamella and threading through the primary and secondary walls, the earlier-formed cell-wall constituents are bound by randomly invading lignin polymers that cross-link via existing FA and PCA ester and ether bonds. This study was conducted to determine the progressive resistance to degradation by differentiating tissues in walls of corn internodes.

Materials and Methods

Growth chamber grown corn at the 15th leaf stage was harvested and Internodes 7 (I7) through 13 (I13) were excised. Internode 6 was the first aboveground internode. Each internode was divided into lower and upper halves, and the outer rind (including cuticle and vascular strands) was separated from the central pith tissue. The pith tissue was comprised mostly of parenchymatous cell types and randomly distributed vascular strands. Ground internode samples were fermented in vitro for 24 and 96 h to provide estimates of the rapidly degradable and potentially degradable fractions, respectively. Neutral sugar residues before and after fermentations were determined by LC after acid hydrolysis of the starch-free, alcohol-insoluble residue. Total uronic acids were estimated colorimetrically using galacturonic acid as the calibration standard. The experiment was in a randomized complete block design with two replicates. Data are presented averaged over location (lower vs. upper) within internode.

Results and Discussion

When harvested I12 and I13 were undergoing rapid cellular elongation in the lower sections, and differentiation and initiation of lignification in upper sections; I11 was still elongating in the lower third of the internode, but had ceased elongation and begun differentiation and lignification in the upper two-thirds of the internode; and I7 through I10 were fully elongated and actively engaged in secondary cell-wall thickening. Degradability of wall polysaccharides declined markedly from the

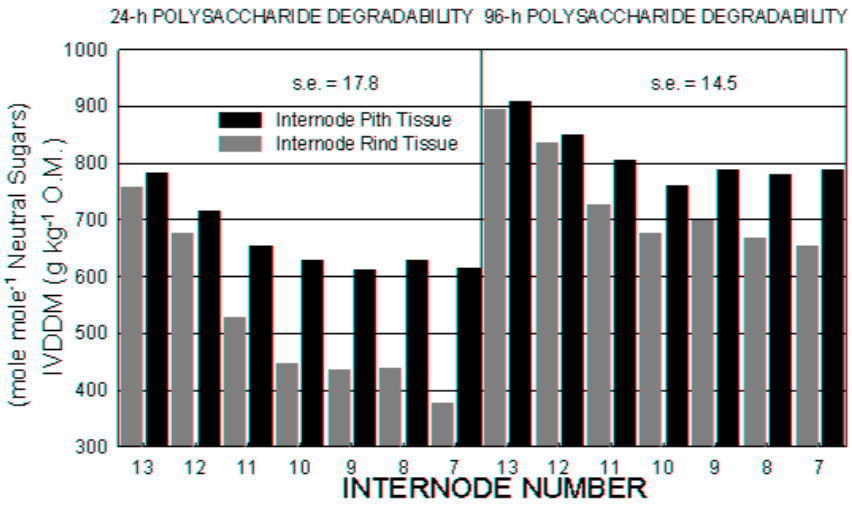


Figure 1. Polysaccharide degradability of pith and rind tissue from internodes of corn after 24- and 96-h incubation in rumen fluid.

youngest internode (I13) through I11 and then leveled off or declined more slowly through the remaining internodes, especially in the pith tissue (Fig.1). During both fermentation times, pith of the younger internodes (I13 to I11) degraded more quickly and extensively than rind; differences in degradability between pith and rind widened with increasing internode maturity. Potentially degradable (96 h) polysaccharide in both pith and rind of I13 was near 90% and remained above 75% in the pith of older internodes, whereas the rind tissue of older internodes degraded only 67%.

Examining the digestibility of the neutral sugars and uronic acids which are the building blocks of wall polysaccarides improves understanding of the manner in which structural carbohydrates interact to limit their digestibility. A substantial increase in degradability occurred between 24 and 96 h for glucose and xylose, with smaller increases in degradability for arabinose, uronic acids and galactose (Table 1). Most of the mannose was degraded by 24 h.

Conclusions

Resistance to digestion increases as soon as lignin polymerization is initiated. It is likely that condensed phenolics in the middle lamella and primary wall of even I13 increase resistance to degradability early in the lignification process. This early resistance is thought to occur from feruloyl-ester cross links between arabinoxylan and the lignin. Degradability remained high in fully differentiated cells of corn pith parenchyma and collenchyma that have secondary wall thickening.

Nonstructural Carbohydrates in Cool- and Warm-Season Perennial Grasses Adapted to Shaded Conditions

K.D. Kephart and D.R. Buxton

Introduction

Forages frequently experience shaded conditions. Shading may occur during periods of cloudiness; from neighboring plants, especially when grown in swards with mixtures differing in height; from companion crops commonly used during seedling establishment; or lower plant parts may be shaded by upper plant parts. Additionally, there is increased interest in incorporating forage systems within agroforestry programs where forages may be shaded by associated trees. Shading has both direct and indirect effects on forage quality in that it can alter morphological development and yield. Cool-season (C_3) species are usually better adapted to shading than warm-season (C_4) species. Generally, growth rates and herbage yield of forages decrease with increasing shade. Morphological plasticity allows plants to maximize growth and to increase persistence under shaded conditions. Exposure to prolonged periods of shade causes most forages to modify proportioning of biomass among plant parts so that the potential for photosynthetic active radiation interception is maintained or increased and root growth is decreased. Shading often reduces tillering; however, stem length is often greater for plants adapted to shade. Shaded grass leaves typically are longer and thinner than when grown in full sunlight.

Shading usually has a smaller effect on forage quality than on morphology or yield. Nitrogen concentration is much more responsive than other quality characteristics. Shading usually increases N concentration substantially, especially in leaves. Most studies have also reported that shading decreases cell-wall concentration of forages. The hemicellulose fraction may be less sensitive to shading than are cellulose and lignin fractions. There is limited information about the effect of prolonged shading on nonstructural carbohydrates of forages. This study was conducted to compare the response of perennial C_3 and C_4 grass species to prolonged shading.

Materials and Methods

Four grass species, differing in photosynthetic type, were grown under three levels of solar radiation. The C_3 species were 'Kentucky 31' tall fescue and 'Vantage' reed canarygrass and the C_4 species were 'Cave-in-Rock' switchgrass and 'Kaw' big bluestem. Polypropylene fabric shades were suspended about 1 m above the soil surface to impose shade treatments of 37 and 70% sunlight beginning in early May during the 2 years of study. A nonshaded control received 100% sunlight. In late May or early June the first of three samplings was taken. Subsequent sampling occurred at 3-week intervals. Freeze-dried samples were solubilized and hydrolyzed for determination of nonstructural carbohydrates. Fractions were collected of water-soluble carbohydrates (monosaccharides), acid-extractable carbohydrates (sucrose plus short-chain fructosans), and α-amylase soluble carbohydrates (starch and other long-chain polymers). Sugar concentrations as glucose/fructose equivalents were determined by using an autoanalyzer. The three fractions were totaled and identified as total nonstructural carbohydrates (TNC).

Results and Discussion

Plants adapted to shade had lower TNC concentrations than those adapted to full sunlight, although differences were not statistically significant for nonlaminar herbage (stems and sheaths) of big bluestem and switchgrass (Fig. 1). The response was much greater in the two C_3 grasses (reed canarygrass and tall fescue) than in the C_4 grasses (big bluestem and switchgrass). Approximately one-third of the TNC was water soluble. Water-soluble carbohydrate concentration was less sensitive to shading than total nonstructural

carbohydrates. In fact, the response in switchgrass, and nonlaminar herbage of reed canarygrass and tall fescue was nonsignificant. Acid-extractable carbohydrates also comprised about one-third of the TNC. Here the response was not significant for switchgrass or big bluestem but was significant for reed canarygrass and tall fescue. Shading reduced the α-amylase soluble carbohydrates only in the nonlaminar herbage of reed canarygrass and tall fescue.

Conclusions

Nonstructural-carbohydrate concentration was much more sensitive to shading in the two C_3 species than in the C_4 species. The greater plasticity in TNC concentration of cool-season species to shading than in warm-season species may be part of the mechanism that allows cool-season species to be better adapted to prolonged shading.

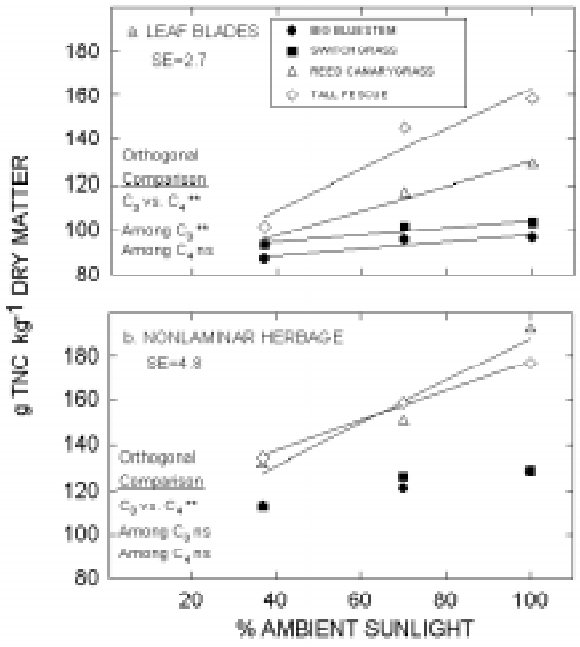


Figure 1. Total nonstructural carbohydrates in C_3 and C_4 perennial grass species adapted to different levels of sunlight

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Alfalfa Cultivar Variation for Minerals Concentration

J. Guan, D.R. Buxton, J.P. Goff and R.L. Horst

Introduction

Milk fever (parturient paresis) is a complex metabolic disorder that occurs at the beginning of lactation in many high-producing dairy cattle. The disease is characterized by a rapid decline in plasma Ca because of the demand for Ca to form colostrum in the milk. Severe hypocalcemia is thought to account for most of the clinical symptoms. If left untreated, most animals die. It is a greater problem in older cows than in heifers. Of the various methods used to control the disease, the most promising is dietary management of dry cows before parturition. Until recently, most attention has centered on manipulating dietary Ca. Recent work has focused on the cation-anion difference (CAD) in the diet as the factor determining the susceptibility of cows to milk fever. The CAD is thought to influence milk fever by affecting blood pH. A reduction in blood pH can be achieved by adding anions or by reducing the concentration of cations in the diet.

Horst et al. recently proposed that CAD be calculated as follows:

CAD (meq kg^{-1}) = (0.38 meq Ca^{++} kg^{-1} + 0.25 meq Mg^{++} kg^{-1} + meq Na^{+} kg^{-1} + meq K^{+} kg^{-1}) - (meq Cl^{-} kg^{-1} + 0.6 meq $SO4^{-}$ kg^{-1} + 0.5 meq $PO4^{---}$ kg^{-1})

This equation assumes that Na, K, and Cl are absorbed with 100% efficiency and that the remaining cations and anions are absorbed at lower efficiencies as shown by their coefficients. Addition of anions to the diet to reduce dietary CAD is a limited option because of reduced palatability associated with anionic salt sources commonly used. A more practical solution may be to reduce cations in the diet. Because of the high efficiency of absorption, recent work has focused on reducing K and Na. This study was conducted to determine if variation exists among alfalfa cultivars for cation mineral concentration and to determine the relationship between concentrations in leaves and stems.

Materials and Methods

Twenty alfalfa cultivars, representing five of the nine original germplasm introductions to the USA, were evaluated for mineral concentrations. Samples for this study were collected in 1993 and 1994. The alfalfa was harvested three times each year, but samples from only the first two harvests were studied. Maturity effects were controlled by selecting stems with only one raceme that had blooming florets. Leaves were separated from stems. Minerals concentration was determined by an atomic absorption spectrophotometer.

Results and Discussion

Significant cultivar variation for all minerals occurred in leaves, stems, and total forage (Tables 1 and 2) with the greatest variation for Na concentration. Results are shown here for only K and Na (Tables 1 and 2). There were some compensating effects so that cultivars low in one mineral were sometimes high in another. When milliequivalents of the cations were totaled according to the equation by Horst et al., the range was 724 meq kg^{-1} for 'Ramsey' to 794 meq kg^{-1} for 'ICI 645.' Often variation from environmental factors was larger than that for cultivars. For example, K concentrations were markedly higher in 1993 (20.2 g kg^{-1}) than in 1994 (17.6 g kg^{-1}) and higher in Harvest 2 (21.2 g kg^{-1}) than in Harvest 1 (16.6 g kg^{-1}). Ca concentrations were higher in Harvest 1 (12.7 g kg^{-1}) than in Harvest 2 (10.0 g kg^{-1}).

Conclusions

While variation exists among these diverse cultivars for minerals concentration, the range may not be great enough to have a meaningful effect on milk fever of cattle. We have yet to determine the anions of these samples. Other

factors such as soil mineral concentration, crop maturity, and environmental conditions during crop growth may be more important in lowering cation concentration in alfalfa.

Table 1. Potassium concentration in leaves and stems of alfalfa cultivars. Data are averaged over two years and two harvests.

Cultivar	Stems	Leaves	Total forage
	- - - - - - - g kg^{-1} - - - - - - - -		
ABI 9144	18.2	20.9	19.7
Archer	17.3	20.5	18.9
Caliverde	16.5	20.9	18.7
Cherokee	16.7	18.3	17.5
Deseret	16.7	21.2	19.1
Gladiator	17.5	19.1	18.4
ICI 645	19.0	21.7	20.4
Iroquois	17.7	20.1	19.0
Norseman	17.5	18.9	18.3
Pioneer 5246	17.4	20.5	19.0
Pioneer 5364	17.8	20.0	19.0
Pioneer 5683	16.3	18.4	17.4
Quantum	18.9	20.8	19.9
Ramsey	18.2	19.3	18.8
Sarnac	17.8	21.2	19.5
Sarnac AR	18.0	21.0	19.5
Valor	17.8	19.0	18.4
WL 222	18.0	20.1	19.1
WL 322 HQ	17.6	20.7	19.2
Washoe	17.1	19.9	18.6
LSD(0.05)	0.91		1.11
Mean	17.6	20.0	18.9
Minimum value	16.3	18.3	17.4
Maximum value	19.0	21.7	20.4

Table 2. Sodium concentration in leaves and stems of alfalfa cultivars. Data are averaged over two years and two harvests.

Cultivar	Leaves	Stems	Total forage
	- - - - - - - - - g kg^{-1} - - - - - - - - -		
ABI 9144	0.397	0.375	0.385
Archer	0.363	0.547	0.459
Caliverde	0.382	0.607	0.497
Cherokee	0.733	0.843	0.790
Deseret	0.169	0.256	0.238
Gladiator	0.360	0.456	0.413
ICI 645	0.388	0.398	0.393
Iroquois	0.230	0.328	0.283
Norseman	0.134	0.362	0.254
Pioneer 5246	0.348	0.396	0.373
Pioneer 5364	0.351	0.493	0.430
Pioneer 5683	0.316	0.612	0.461
Quantum	0.254	0.323	0.290
Ramsey	0.294	0.331	0.315
Sarnac	0.334	0.432	0.385
Sarnac AR	0.461	0.501	0.482
Valor	0.236	0.418	0.331
WL 222	0.224	0.422	0.329
WL 322 HQ	0.258	0.451	0.356
Washoe	0.220	0.460	0.348
LSD(0.05)	0.063		0.048
Mean	0.323	0.451	0.391
Minimum value	0.134	0.256	0.238
Maximum value	0.733	0.843	0.790

Rumen Microbiology

The Effect of pH on Ruminal Methanogenesis

J.S. Van Kessel and J.B. Russell

Introduction

Methane is a major end product of ruminal fermentation and, in cattle, methane losses can represent as much as 10% of the dietary energy. Ruminal methanogenesis represents an alternative mechanism of reducing equivalent disposal for carbohydrate-fermenting bacteria, but interspecies hydrogen transfer is only exergonic at very low partial pressures of hydrogen. If the methanogens are inhibited, hydrogen accumulates, the hydrogenases are inhibited, and the carbohydrate-fermenting bacteria utilize other mechanisms of reducing equivalent disposal (e.g., the dehydrogenases of propionate production). It has long been recognized that the addition of cereal grains to ruminant diets causes a decrease in methane and an increase in propionate production, but the cause of this fermentation shift was not clear. Some starch-fermenting ruminal bacteria produce propionate, but starch feeding can also cause a decrease in ruminal pH and a marked shift in the numbers of other ruminal bacteria. Despite the fact that ruminal methanogenesis is a key determinant of fermentation stoichiometry, the effect of pH on ruminal methanogens had not been examined previously.

Materials and Methods

Rumen contents were obtained from two ruminally cannulated, mature Holstein cows. The cows were housed in a tie-stall barn, fed twice daily and provided with water ad libitum. The cows were fed either a forage diet (timothy hay) to provide a high ruminal pH or a concentrate diet (corn and soybean meal) to induce a low pH ruminal environment. Ruminal pH was monitored at 2 hour intervals. Ruminal contents were collected 3 h post-feeding and pH was determined immediately. The ruminal contents were incubated anaerobically at 39 °C for 30 min. Bacteria were collected from the middle section of the flask. Ruminal fluid was anaerobically diluted 10-fold in a basal medium containing salts, cysteine hydrochloride, Na_2S, vitamins, microminerals, mercaptoethanesulfonic acid, a volatile fatty mixture, Trypticase, and yeast extract. When acetate concentrations were reduced from 100 mM to as low as 0 mM, sodium acetate was replaced with sodium chloride. The medium containing ruminal microorganisms was anaerobically transferred to serum bottles. One atmosphere of hydrogen gas was added with a hypodermic syringe. The bottles were incubated at 39 °C. Head space samples were removed, and methane was measured with a gas chromatograph. Volatile fatty acids in cell and particle-free ruminal fluid were analyzed by high-pressure liquid chromatography.

Results

When a fistulated cow was fed an all forage diet, ruminal pH remained more or less constant (6.7 to 6.9). The ruminal pH of a concentrate-fed cow decreased dramatically in the period soon after feeding, and the pH was as low as 5.45. Mixed ruminal bacteria from the forage-fed cow converted CO_2 and H_2 to methane, but the ruminal fluid from the concentrate-fed cow did not produce methane. When the pH of the ruminal fluid from the concentrate-fed cow was adjusted to pH 7.0, methane was eventually detected, and the absolute rate constant of methane production was as high as the one observed with ruminal fluid from the forage fed

cow (0.32 h^{-1}). Based on the zero-time intercepts of methane production, it appeared that the concentrate-fed cow had fewer methanogens than the forage-fed cow. When the mixed ruminal bacteria were incubated in a basal medium containing 100 mM acetate, methanogenesis was pH-dependent, and no methane was detected at pH values less than 6.0. Because the removal of acetic acid completely reversed the inhibition of methanogenesis, it appeared that volatile fatty acids were causing the pH-dependent inhibition.

Discussion

Pure cultures of ruminal bacteria often produce end products, such as ethanol, that are not detected in the rumen; and methanogens, by consuming hydrogen, can alter the profile of fermentation end products in mixed culture environments like the rumen. When reducing equivalents are transferred from carbohydrate-fermenting ruminal bacteria to methanogens, acetate increases and propionate generally declines. Hydrogen and carbon dioxide are the primary sources of methane in the rumen, and acetate is not a significant precursor for ruminal methane.

The methane production of cattle is relatively difficult to measure, but methane can be estimated from the stoichiometry of the fermentation acids. Based on fermentation acids alone, the methane production of the forage-fed cow would have been 1.5-fold greater than the concentrate-fed cow:

100 glucose $\rightarrow$ 134.5 acetate + 31.9 propionate + 16.8 butyrate + 31.9 H_2O + 100.8 CO_2 + 67.2 CH_4,

versus

100 glucose $\rightarrow$ 9.9 lactate + 93.2 acetate + 52.2 propionate + 22.4 butyrate + 48.4 H_2O + 93.2 CO_2 + 44.7 CH_4.

These balances, however, do not consider the fact that bacterial cells are also end products of ruminal fermentation. Because microbial cells have a negative oxidation-reduction state, cell production can have a significant impact on the estimate of methane production. Based on an empirical formula of $C_{1.00}H_{1.58}O_{0.77}$ and an oxidation-reduction state of -1.69, and a yield of 30 g cells per 100 g carbohydrate fermented, the methane estimate was considerably lower and the difference between forage-fed and concentrate-fed cows was greater:

100 glucose $\rightarrow$ 94.1 acetate + 22.4 propionate + 11.8 butyrate + cells [$C_{180}H_{284}O_{85}$] + 94.1 H_2O + 82.4 CO_2 + 35.3 CH_4

versus

100 glucose $\rightarrow$ 7.0 lactate + 65.2 acetate + 36.5 propionate + 15.7 butyrate + cells [$C_{180}H_{284}O_{84}$] + 86.0 H_2O+ 87.0 CO_2 + 9.6 CH_4 + 29.1 H_2

Based on these latter stoichiometries, the concentrate-fed cow was producing some methane, but ruminal fluid obtained 3 h after feeding had a pH of 5.4 and did not produce methane, even if hydrogen was added. The inhibitory effect of low pH was supported by a variety of observations: 1) the addition of base to the ruminal fluid from the concentrate-fed cow allowed methane production, 2) the zero-time intercept of methane production indicated that the concentrate-fed cow had fewer (or less active) methanogens than the forage-fed cow, and 3) bacteria from the forage-fed cow could not produce methane if the pH was less than 6.0.

The toxicity of acetate at low pH has been explained by the influx of acid and metabolic uncoupling, but this theory does not explain why some bacteria are inhibited and others are not affected. When the internal pH is more alkaline than the external pH (ΔpH), volatile fatty acids

will cross the cell membrane, dissociate and accumulate intracellularly. Because the volatile fatty acid concentration of ruminal fluid is relatively high, an even modest increase in ΔpH can lead to a dramatic increase in intracellular volatile fatty acid anions. The intracellular pH regulation of methanogenic bacteria has not been examined in a systematic fashion, but some methanogens have large pH gradients across their cell membranes at acidic pH values.

Methane is a "greenhouse gas," and methane accumulation in the atmosphere has been cited as a factor in global warming. Methane appears to have as much as a 60-fold higher global warming potential than carbon dioxide. Ruminants have always been included as significant producers of atmospheric methane, but their overall contribution has been a lively subject for debate. The pH-dependence of ruminal methanogens provides a basis for predicting methane production. Because poor quality forage diets never cause a significant decrease in ruminal pH, these diets should always produce large amounts of methane. High quality, concentrate diets have the potential to decrease methane production, but only if the intake is high enough to cause a reduction in ruminal pH.

Effect of Forage Quality and Monensin on the Ruminal Fermentation of Fistulated Cows Fed Continuously at a Constant Intake

R.P. Lana and J.B. Russell

Introduction

Monensin improves the feed efficiency of ruminants, but decreases in methane and increases in propionate cannot explain all of this benefit. Early research indicated that monensin decreased ruminal ammonia in vivo, and this finding was corroborated by mixed culture and pure culture studies. The amino acid-sparing effect of monensin has not been well documented. Monensin increased passage of feed protein, but this increase was offset by decreases in bacterial protein. In some cases, monensin had no effect on ruminal ammonia, and it appeared that monensin-dependent improvements in nitrogen retention were a result of energy utilization rather than the protein sparing per se. Previous work indicated that cattle fed timothy hay and soybean meal 12 times per day had steady-state ruminal fermentation patterns; and, under these conditions, monensin decreased ammonia concentration, specific activity of deamination and most probably the number of obligate amino acid-fermenting bacteria. Preliminary experiments indicated that cattle fed alfalfa hay did not respond in a similar fashion, and the present research was designed to examine the relationship between forage quality and monensin-dependent amino acid sparing.

Materials and Methods

Two nonlactating cows (685 ± 59 kg) fitted with ruminal cannulas were fed forage 12 times per day. Dietary treatments consisted of three combinations of timothy and alfalfa hays (100:0, 50:50, and 0:100) and two levels of monensin (0 and 350 mg cow^{-1} d^{-1}). Monensin (350 mg) was dissolved in 36 mL of ethanol, and 3 mL of the solution were added on the top of each meal. Water was offered free choice. No salt or other minerals were provided.

Ruminal fluid (500 ml) was squeezed through four layers of cheesecloth and analyzed for pH with a combination electrode. Volatile fatty acids in cell-free samples were measured by high-performance liquid chromatography. Ammonia in cell-free supernatant fluid was measured by colorimetry. Ruminal K and Na concentrations in cell-free supernatant fluid were determined by flame photometry. Bacterial protein was assayed by the method of Lowry. Bacterial pellets were digested with 3 *N* HNO_3 (room temperature, 24 h) before analyzing for K by flame photometry.

Separate samples of ruminal fluid were collected from the rumen and allowed to incubate anaerobically in a 39 °C water bath for 60 min. Feed particles were buoyed to the top of the flask by gas production and protozoa sedimented to the bottom of the flask. Mixed ruminal bacteria (10 mL) from the center of the flask were transferred in triplicate to 15 mm x 180 mm tubes. At time zero, an anaerobic solution of Trypticase was added to the tubes (15 g/L final concentration). The tubes were incubated at 39 °C for 6 h. The incubation was terminated by a centrifugation step (10,000 x g, 20 min, 0 °C) that removed the bacteria. The bacterial pellet was washed with .9% NaCl (wt/vol) and stored at -15 °C. The cell-free supernatant fluid was stored separately at -15 °C. Ammonia and bacterial protein were measured as described previously. Preliminary experiments indicated that ammonia production was first order with respect to time and cell concentration so long as the incubation period was less than 10 h.

Cows (n = 2) were the experimental units for the statistical analysis. Each experimental unit was the mean of pooled data from four sample units (4 d of collection). All statistical analyses were performed with the GLM procedures of Minitab. Data were analyzed as a randomized complete block design. Sources of variations included animals (blocks), alfalfa (0% vs. otherwise), alfalfa level (50 vs. 100% alfalfa), monensin, alfalfa x monensin, and alfalfa level x monensin. The residual error term (block x treatment

effects) was used for testing treatment effects and interactions at a = .05. If the F test indicated that a model interaction was significant ($P < .05$), interactive treatment means were analyzed by the method of Tukey.

Results

The alfalfa hay had 1.4 times less NDF than the timothy hay, and the concentration of VFA in the ruminal fluid was greater ($P < .05$) when alfalfa was substituted for timothy. The substitution of alfalfa for timothy did not affect ruminal pH or the acetate:propionate ratio ($P > .05$). Alfalfa decreased bacterial protein in the fluid phase ($P < .05$). The alfalfa had 1.5 times as much K as the timothy hay. Alfalfa increased ($P < .001$) the K concentration (K / [K + Na]) of the ruminal fluid and decreased the K content of the bacteria ($P < .05$). The alfalfa had 1.4 times as much CP as the timothy hay, and total ruminal ammonia increased ($P < .05$) when alfalfa was substituted for timothy (Table 2). Dissociated ammonia, as predicted from pH and the Henderson-Hasselbalch equation ($pH = pK_a + \log [NH_3]/[NH_4^+]$), was unaffected by alfalfa substitution ($P > .05$). Substitution of timothy with alfalfa hay increased ($P < .001$) the specific activity of deamination (deamination rate).

At all combinations of timothy and alfalfa hays, monensin caused an increase ($P < .001$) in total VFA and a decrease ($P < .05$) in ruminal pH. Acetate increased to a smaller extent than propionate, but the decrease in acetate:propionate ratio was only significant ($P < .05$) when alfalfa was 50% or less of the forage. Monensin caused a small but significant ($P < .001$) increase in the K concentration of the ruminal fluid, but it did not have an effect on the K content of bacteria in ruminal fluid ($P > .05$). Monensin did not decrease total ammonia when alfalfa was 0 or 50% of the diet and even increased it in 100% alfalfa. The total ammonia, however, was not corrected for changes in ruminal pH. Monensin decreased ($P < .05$) the dissociated ammonia concentration when only timothy hay was fed, but monensin had no impact ($P > .05$) on dissociated ammonia if alfalfa was 50 or 100% of the diet. Monensin alleviated alfalfa-dependent decreases in bacterial protein ($P < .05$) and decreased ($P < .001$) the specific activity of deamination (deamination rate) at all combinations of alfalfa and timothy.

Discussion

Because monensin did not decrease total ammonia accumulation when animals were fed 100% alfalfa, it seemed that some component in alfalfa was counteracting potential decreases in deamination. Because monensin catalyzes the efflux of K from monensin-sensitive ruminal bacteria, high ruminal potassium may inhibit monensin action. Our animals were not given supplemental Na, and the K ÷ (K + Na) values were high (.56 to .68), and animals fed alfalfa had a greater K intake and higher ruminal K (K ÷ [K + Na]) than animals fed only timothy.

Animals fed monensin had lower ruminal pH values than untreated controls, and these differences could have influenced the absorption rate of ammonia from the rumen. Dissociated ammonia is a much more lipophilic substance than ammonium ion, and it would be more rapidly absorbed. Based on ruminal pH and the pK_a of ammonia, it was possible to estimate the ruminal concentration of dissociated ammonia. Because monensin decreased steady-state dissociated ammonia when timothy was 100% of the diet, it seemed that monensin had the potential to spare amino acids from deamination. Monensin did not decrease dissociated ammonia when alfalfa was fed, but it was able to alleviate alfalfa-dependent decreases in bacterial protein.

The observation that monensin decreased the specific activity of deamination at all combinations of alfalfa and timothy further supports the idea that monensin had the potential to spare amino acids. The effect of monensin on deamination is consistent with its ability to inhibit Gram-positive, highly active, obligate amino acid-fermenting ruminal bacteria in vitro and in vivo. Monensin is clearly an antimicrobial substance, but ruminal VFA concentrations were

always greater when monensin was added to the diet. Increases in ruminal VFA were correlated with the increases in bacterial protein. Monensin (across all three diets) caused a 20% increase in total VFA and a 20% increase in bacterial protein in the fluid phase. This comparison indicated that: 1) monensin enhanced VFA production, 2) the bacteria had more ATP for growth, and 3) the animal would have more bacterial protein.

The rumen often has a dense population of protozoa, but protozoal counting procedures are confounded by the propensity of protozoa to associate with feed particles and by the difficulty in obtaining representative samples. In vitro results indicated that protozoa were sensitive to monensin, but this effect has been difficult to document in vivo. Because ruminal protozoa store large amounts of carbohydrate as intracellular glycogen, appear to recycle bacterial nitrogen, and decrease microbial flow from the rumen, it is conceivable that a monensin-dependent decrease in protozoa caused the increase in bacterial protein and VFA that we observed.

The Lysis of *Fibrobacter Succinogenes*

J.E. Wells and J.B. Russell

Introduction

In ruminant animals, microbial protein is the primary source of protein for the animal, and microbial protein turnover is a wasteful process that decreases amino acid availability. Protozoal predation has often been cited as the prime cause of bacterial protein turnover in the rumen, but high rates of bacterial turnover have been noted in defaunated sheep. Bacteria need autolytic enzymes to expand their cell wall and grow, but the cells have a very high turgor pressure. If the action of autolytic enzymes is not carefully orchestrated, the cells can lyse. Because *Fibrobacter succinogenes* is a ruminal bacterium that lyses easily, it makes a good model for studying rumen bacterial turnover.

Materials and Methods

Fibrobacter succinogenes S85 was grown anaerobically in a minimal medium that contained cellobiose. Cultures were grown (39 °C) in 80 ml serum bottles, 18 x 150 mm tubes or a continuous culture device. Growth and lysis were monitored by the changes in optical density as well as changes in cell protein. Protein was determined by the Lowry method. RNA and DNA were determined using orcinol and diphenylamine methods, respectively. Cell N was calculated from protein, RNA and DNA with the N content of protein and nucleic acids being 16 and 12%, respectively. The membrane potential ($\Delta\Psi$) was calculated from the uptake of [^{3}H] TPP^+ using the Nernst equation (62 mV x log [in]/[out]), and the non-specific binding of [^{3}H] TPP^+ was estimated from cells which had been treated with nigericin plus valinomycin (10 mM each) or toluene (1% of a 1:9 [v/v] toluene to ethanol). ATP was assayed with a luminometer. Cellobiose and cellular polysaccharide were assayed by an anthrone procedure. Soluble protein was measured by the Bradford dye method. Ammonia was assayed by a calorimetric method. Free amino acids were measured by high pressure liquid chromatography. Peptides were hydrolyzed with HCl prior to derivatization and amino acid analysis.

Results

Growing cultures of *F. succinogenes* assimilated more ammonia than could be accounted by cellular protein, RNA or DNA, and released large amounts of non-ammonia nitrogen. The difference between net and true growth was most dramatic at low dilution rates, but mathematical derivations indicated that the lysis rate was a growth rate-independent function. The lysis rate was 7-fold greater than the true maintenance rate (0.07 h^{-1} versus 0.01 h^{-1}). Because slow-growing cells had as much protonmotive force and ATP as fast-growing cells, lysis was not a starvation response per se. Stationary cells had a lysis rate that was 10-fold less than growing cells. Rapidly-growing cells were not susceptible to the proteinase inhibitor, PMSF, but PMSF increased the lysis rate of the cultures when they reached stationary phase. This latter result indicated that autolysins of stationary cells were being inactivated by a serine proteinase. When growing cells were treated with the glycolytic inhibitor, iodoacetate, the proteinase-dependent transition to stationary phase was circumvented, and the rate of lysis could be increased by as much as 50-fold.

Discussion

Lysis is usually considered to be a property of stationary phase bacteria that have depleted their nutrients, but *F. succinogenes* had a much faster rate of lysis when it was growing. In the surface stress model of bacterial growth, peptidoglycan is first deposited at the inner surface, and the older, outer layers are then cut by autolytic enzymes. This continual process of synthesis and degradation allows the stress to be gradually transferred to more recently synthesized portions

of the peptidoglycan. Since each new layer of the peptidoglycan is slightly longer than the preceding one, the wall is continually expanded.

Some workers hypothesized that the autolysins might be regulated by protonmotive force. In this model of autolytic regulation, protonmotive force decreases pH near the cell membrane, and acidic pH prevents the autolysins from completely degrading the peptidoglycan. Uncouplers that decreased protonmotive force promoted the lysis *of F. succinogenes*, but lysis and protonmotive force could not always be correlated. Growing cells with a high protonmotive force lysed, and stationary-phase cells that had a low protonmotive force did not lyse very rapidly.

Stationary cells of *F. succinogenes* had a mechanism of preventing lysis, and the experiments that included PMSF indicated that a proteinase was involved in autolysis. Because PMSF had no effect on exponentially growing *F. succinogenes* cultures but promoted lysis when the cells reached stationary phase, it appeared that a serine proteinase was inactivating the autolysins when growth was no longer possible. One might view the lysis of growing *F. succinogenes* cells as a detrimental phenomenon that would decrease its niche in the rumen, but this assumption does not address the aspect of maximum growth rate and cell division. If autolytic activity is too low, growth rate would be sacrificed.

If the autolysis and growth of *F. succinogenes* are indeed highly integrated processes, lysis may be a very difficult phenomenon to alter. Lysis is, however, a dynamic process that must compete with the fluid dilution rate. Mineral salts have no direct effect on growth or lysis per se, but they increase the ruminal fluid dilution rate. When fluid dilution rate is increased, fluid phase bacteria have less time to lyse and turn over.

Variation in Ruminal and Milk Parameters Among Cow-Diet Combinations: Results From a Baseline Study to Relate Digestion Kinetics and Microbial Population Data

P.J. Weimer, G.M. Waghorn and D.R. Mertens

Introduction

Despite the central importance of the ruminal microflora in the digestion of feedstuffs by ruminants, there have been no systematic studies of the quantitative relationship between particular ruminal microbial species and ruminal chemistry (e.g., pH, VFA concentrations), digestion kinetics (e.g., lag, rate, and extent of digestion) and animal performance (e.g., milk production and milk composition). As part of a larger study aimed at relating microbial populations to these parameters, we examined the variation in VFA patterns among diets in four cows that were used in an experiment for determining digestion kinetics (using both in vitro and in situ methods) and for assessing microbial populations (using oligonucleotide probes directed toward species-specific ribosomal RNA molecules).

Methods

Four multiparous mid-lactation Holstein cows were fed, in a Latin-square design, diets differing in source (corn silage or alfalfa silage) and amount (24% or 32% NDF) of fiber. Diets were offered at 12 h intervals as isonitrogenous total mixed rations in amounts that assured ad libitum intake. After adaptation to diet for 19 days, 13 samples were collected from each rumen over a 4-day period; Dacron bags containing various feedstuffs were also removed from each rumen over the 4-day period to determine in situ digestion kinetics. Samples were analyzed for pH and VFAs and were stored at -80 ^{o}C for subsequent analysis of microbial populations. VFAs were determined by HPLC. Rumen fluid was also collected at the beginning and end of the four day period and were used as inocula for determination of in vitro digestion kinetics.

Results

Cows displayed marked differences in ruminal pH determined just prior to feeding (range of means [n = 3], 5.36 to 6.49) or 3h post-feeding (range of means [n = 5], 5.04 to 5.94), but there was no significant relationship between the pre- and post-feed pH values (r^2 = .136). Despite the low pH of many of the ruminal samples, none of the 208 samples examined in this study contained significant concentrations of lactate or succinate.

Molar concentrations of ruminal VFAs (acetate, propionate, butyrate, and the sum of these acids) were remarkably constant over the 12-h feeding cycle. Individual cows displayed marked variation in the amounts and proportions of VFAs, and marked variations were observed among diets within cows as well (Fig. 1). Ruminal acetate/propionate (A/P) ratios (Fig. 2) and milkfat (Fig. 3) varied markedly among cow-diet combinations. The positive correlation (r^2 = .73) between milkfat and A/P ratio was even stronger (r^2 = .88) at high (> 100 mM) acetate concentrations. Milkfat was not correlated with concentration of acetate (r^2 =.002) or butyrate (r^2 = .07) but was negatively correlated with propionate concentrations (r^2 = .50). In general, diets based on alfalfa silage yielded higher A/P values and higher milkfat compositions than did corn silage-based diets at equivalent NDF concentrations.

Conclusions

Large, statistically-significant differences in ruminal pH, VFA profiles, and milk parameters were observed among the four cows and among diets within cows. These data will provide a valuable set for relating ruminal and milk

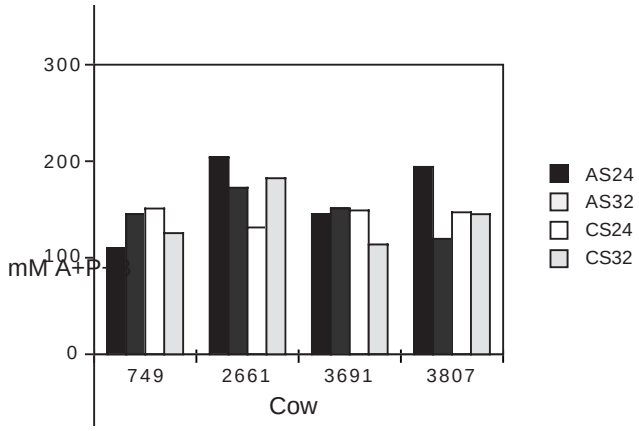

Figure 1. Mean values of the sum of the ruminal concentrations of acetate, propionate, and butyrate in four cows fed four different diets.

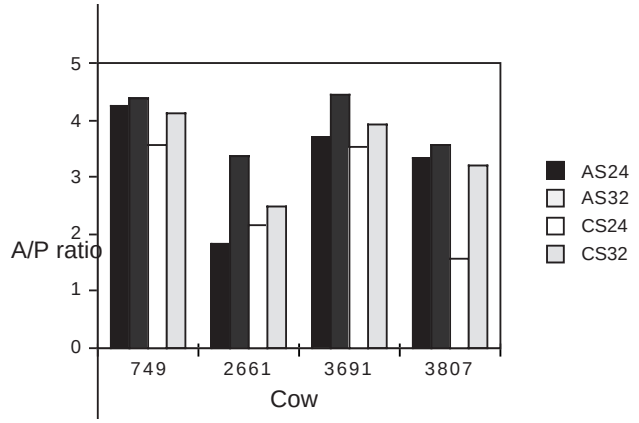

Figure 2. Mean values of ruminal acetate/propionate ratio.

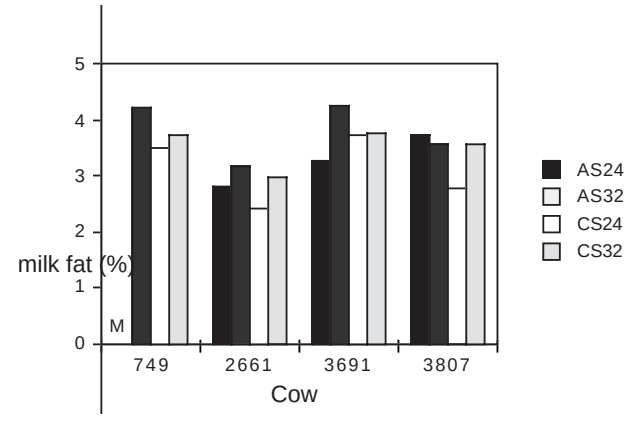

Figure 3. Mean values of fat content of milk. M indicates lack of data for cow 749 on the AS24 diet due to development of mastitis at the end of experimental period 4.

parameters to digestion kinetics and to specific populations of ruminal microorganisms, permitting a systematic determination of the relationships among ruminal chemistry, ruminal microbial populations, digestion kinetics, and animal performance.

Time-Dependent Shifts in Fermentation Endproduct Ratios: A Source of Bias in Estimation of Digestion Kinetics Via In Vitro Measurement of Gas Production

P.J. Weimer

Introduction

Continuous online measurement of gas production in sealed vials has the potential to determine, with greater ease than conventional methods, the kinetics of fermentation of forage materials by ruminal microorganisms. As a result, gas production has been used to compare in vitro kinetics of digestion of various feedstuffs. However, direct calculation of substrate consumption from gas production data is not feasible for a number of reasons. For example, differences in the composition among feeds result in different ratios of fermentation endproducts, which, in turn, affects gas production. A relationship between gas yields and fermentation endproducts has been described by Beuvink and Spoelstra:

mL gas production = V_m [acetate] + 2(V_m) [butyrate] + 0.87 V_m [total VFA], [1]

where V_m is the molar gas volume; these workers routinely measure gas production at the end of the fermentation to provide qualitative information on the deviation of observed gas production from theoretical values.

Digestion kinetics based on gas production can be biased by changes in the proportions of fermentation endproducts that may accompany digestion in batch culture. The purpose of this study was to determine the extent of change in the formation of endproducts during fermentation of three substrates and to quantify the extent to which these changes can bias the theoretical gas yields from a fixed amount of substrate over the course of an in vitro digestion experiment.

Methods

Concentrations of VFAs were determined over time during the fermentation of three substrates: corn silage, alfalfa hay, and microcrystalline cellulose; the last substrate, while not a direct feedstuff for ruminants, is often used as a standard substrate in in vitro gas production experiments. Fermentations were conducted under a CO_2 atmosphere in 60 ml serum vials that contained 80 mg of substrate and 6 ml of a pre-reduced, modified Goering and Van Soest buffer. After warming of vials to 39 °C, vials were inoculated with 4 ml of ruminal inoculum from a cow fed alfalfa hay; the inoculum was prepared by squeezing rumen fluid through four layers of cheesecloth followed by wetting of the solids with an equal volume of Goering/Van Soest buffer and squeezing this through the cheesecloth. Vials were sealed with butyl stoppers and aluminum crimp seals, then incubated at 39 °C. Pairs of vials containing each substrate were removed at intervals, and VFA concentrations determined by HPLC.

Results

The shift in the molar ratios of acetate and butyrate during the fermentations are shown in Fig.1. Shifts in the molar ratios of propionate were also observed but were excluded from the figure because propionate production does not contribute to net production of gas. The molar ratios of products for alfalfa fermentation were

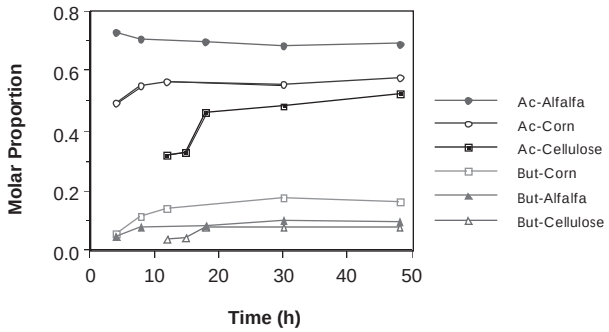

Figure 1. Shifts in the molar proportion of acetate and butyrate during the in vitro fermentation of alfalfa hay, corn, and microcrystalline cellulose.

essentially constant throughout the incubation period. However, both corn silage and microcrystalline cellulose showed marked increases in the molar proportions of acetate and butyrate during the course of the fermentations.

To determine the potential bias in gas production resulting from these time-dependent changes in gas production, the fermentation product data at different times were normalized on a molar basis using a modification of equation [*1*]:

$$Y_t = X_{acetate} + 2\,X_{butyrate} + 0.87 \qquad [2]$$

where Y_t is the relative gas production per unit substrate at time *t* (estimated from equation [*1*]), and $X_{acetate}$ and $X_{butyrate}$ are molar proportions of acetate and butyrate, respectively.

Bias in gas production estimated at different times using fixed, endpoint (48 h) values of VFA proportions were then calculated as:

$$Bias = (Y_t - Y_{48h}) / Y_{48h} \qquad [3]$$

where Y_{48h} is estimated gas production per unit substrate based on the proportions of VFAs at 48 h. A positive value indicates an underestimate of substrate consumption (i.e., an overestimate of gas production relative to that expected for fermentation endproduct ratios determined at the end of the incubation) . Results from these calculations are shown in Fig. 2. Bias introduced by changes in fermentation endproduct ratios was greatest at early time points and reached values of 20% or more. Because measurements during the first 24 h have the greatest effect on determining digestion rate and lag time, changes in VFA patterns during the fermentation must be accounted for to prevent biased estimates of digestion kinetics.

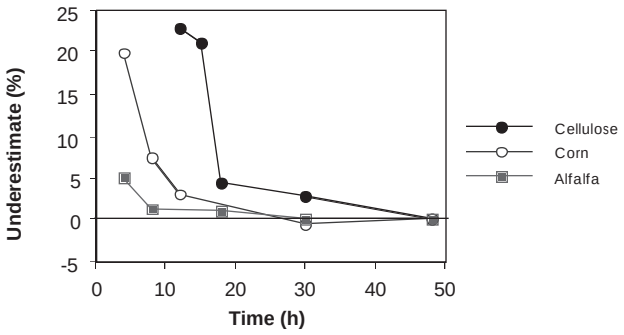

Figure 2. Time-dependent bias in the estimation of digestion of three substrates from gas production measurements. Bias was calculated on the basis of differences in the endproduct ratios at the indicated times relative to the ratios at the 48 h endpoint.

Conclusions

Changes in the ratios of fermentation endproducts over time complicate determination of substrate consumption from gas production data. In this experiment, bias in gas production introduced by assuming fixed endproduct ratios is small for alfalfa but may result in an underestimation of 20% or more for corn and cellulose early in their fermentations. Further experiments are needed to determine if the shifts in fermentation products observed for corn and cellulose were due to characteristics of the feed or to the use of inocula from an alfalfa-fed cow. Nevertheless, proper corrections of gas production data to more accurately reflect substrate consumption will require separate determinations of VFA profiles over time for each substrate of interest.

Inocula Differences Affect In Vitro Fiber Digestion Kinetics

D.R. Mertens, P.J. Weimer and G.M. Waghorn

Introduction

Using digestion kinetics to estimate ruminal responses may provide information useful in formulating dairy rations. However, measurement of kinetic parameters, especially for fiber, may be sensitive to experimental conditions because differences during early fermentation (< 24 h) have a great impact on fractional digestion rates. Thus, factors which cause little difference in 48 h digestion of dry matter may have much greater impact on measurement of kinetic parameters. If digestion kinetics are to be used for feed evaluation and ration formulation, techniques must be established that give accurate and precise parameters. The objective of this project was to evaluate the effects of the animal donor and its diet on the measurement of digestion kinetics. This project was a part of a larger experiment to investigate the effects of fiber source and level on digestion and microbial ecology in the rumen.

Methods

Four cows in midlactation were used in a balanced 4 x 4 Latin square design with a factorial arrangement of treatments (2 fiber sources x 2 fiber levels). Diets containing alfalfa or corn silage, each mixed with corn, soybean meal, and minerals to obtain rations with 24 or 32% amylase-treated NDF (aNDF), were fed twice daily during the four-week periods. After three weeks, ruminal contents were collected from each cow, blended with chilled buffer to detach bacteria, and used to inoculate flasks containing either alfalfa or corn silage. The in vitro method of Goering and Van Soest (1970) was modified so fermentations were terminated after 0, 3, 5, 9, 15, 24, 30, 36, 48, 72, or 96 h. During the last week of each period, the average pH at 3 h post-feeding was also determined for each cow. At the end of the fourth week, a second in vitro trial was conducted with the pH of the in vitro system adjusted to reflect the pH of the donor animal. Kinetic parameters were determined for a single first-order model with discrete lag time using nonlinear regression.

Discussion

Ruminal pH differed among donors and diets. Cow 2661 had an exceptionally low pH throughout the experiment with an average prefeeding pH of only 5.8. Averaged across all cows, the lowest prefeeding pH of 5.78 and 6.14 were associated with alfalfa (AS24) and corn silage (CS24) diets, respectively, containing 24% aNDF. Alfalfa (AS32) and corn silage (CS32) rations containing 32% aNDF resulted in prefeeding pH of 6.07 and 6.30, respectively. Post-feeding ruminal pH was lowest for CS24 and highest for AS32 (Table 1). Average pH for the standard in vitro method was between 6.46 and 6.56. Average pH of the in vitro trials in which pH was adjusted to match that of the inocula donor was 5.89, 5.76, 5.61, and 5.54 for diets AS32, AS24, CS32, and CS24, respectively, which were within about 0.1 of the post-feeding pH associated with these diets (Table 1).

Digestion kinetics differed between forage sources and between in vitro system pH. In addition, both cow donor and its diet affected digestion kinetics. In general, potentially digestible aNDF decreased, fractional rate of fiber digestion decreased, and indigestible aNDF increased as the ruminal pH of the donor cow or diet decreased (Table 1). The observation that indigestible fiber is not constant across inocula indicates that this kinetic parameter is not simply an intrinsic property of the fiber, but is the result of the interaction between cell wall properties and the microbial population available for fermentation. Associated research in the larger experiment will attempt to define the microbial populations associated with each inocula.

Table 1. Differences in ruminal pH and digestion kinetics associated with donor and diet of the inocula. Data are averaged across forage source, in vitro pH, and period (n = 16).

Effects of inocula donor		Effects of inocula diet	
Post-feeding ruminal pH			
749	5.69[a]	AS32	5.78[a]
3691	5.67[a]	AS24	5.52[b]
3807	5.60[a]	CS32	5.53[b]
2661	5.23[b]	CS24	5.37[c]
Potentially digestible aNDF (% of DM)			
749	20.1[a]	AS32	21.3[a]
3691	18.9[ab]	AS24	19.2[b]
3807	18.5[ab]	CS32	17.8[bc]
2661	18.0[b]	CS24	17.2[c]
Fractional rate of digestion (/h)			
3691	0.059[a]	AS32	0.060
749	0.056[ab]	AS24	0.052
3807	0.054[ab]	CS32	0.052
2661	0.044[b]	CS24	0.048
Indigestible aNDF (% of DM)			
749	19.4[a]	AS32	18.5[a]
3691	20.4[ab]	AS24	20.3[b]
3807	20.7[ab]	CS32	21.2[bc]
2661	21.7[b]	CS24	22.2[c]
Discrete lag time (h)			
749	3.21	AS32	3.55
3691	3.60	AS24	2.99
3807	3.40	CS32	4.21
2661	3.31	CS24	2.76

[a,b,c]Values with different subscripts within a group are different at $P < .05$.

Conclusions

Digestion kinetics are a function of the intrinsic properties of feeds and the microbial population that ferments them. Differences between cows and their diets affected the digestion kinetics of alfalfa and corn silage, and the differences seemed to be associated with ruminal pH. Our results suggest that ruminal pH may play a central role in microbial ecology of the rumen and indicate that accurate modeling of ruminal pH must be a component of any dynamic model of ruminal fermentation using digestion kinetic parameters.

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Feed Utilization by Cattle

Comparing Systems of Ration Formulation for Dairy Cows

D.R. Mertens

Introduction

Several systems are being used to estimate requirements, describe feed nutritive value, and formulate rations for dairy cows. Although these systems have a common objective, the approach, complexity, and relative importance of feed and animal attributes differ. Some aspects of these systems have been compared, such as method of defining energy requirements and feed values or specific aspects of protein utilization (microbial yield, fraction of microbes resulting in absorbed amino acids, digestibility of feed and microbial protein, etc.). However, no attempt has been made to compare systems in total to determine if they obtain similar rations when using the same feed ingredients for cows with the same characteristics.

Methods

Four systems were compared: National Research Council using absorbed protein (NRC, 1989); Net Carbohydrate-Protein System (NCPS, Sniffen et al., 1992); Institut National de la Recherche Agronomique (INRA, Jarrige, 1989); and NDF-Energy Intake/Absorbed Protein (NDF/AP, Mertens, 1992, Mertens and Dado, 1993). Using each system, rations were formulated for two multiparous cows (66 months of age), but only the data for the cow in the 10th week of lactation with a body condition score of 3.0, weighing 630 kg, losing .02 kg of weight per day, and producing 43 kg of milk per day containing 3.48% fat and 2.97% protein are presented.

For each cow and system, four rations were formulated using grass hay or alfalfa, corn, or grass silages as the sole forage. Forages were selected for each system that had similar chemical composition. Ground corn, soybean meal (44%CP), and minerals (salt, calcium carbonate, and monosodium phosphate) were the primary concentrates, but fish meal (low degradable protein source), urea (high solubility nitrogen source), animal fat (energy source), and cottonseed hulls (fiber source) were included as potential supplements to meet the requirements of some systems.

Solutions to the NRC and NDF/AP systems were obtained using linear programming with minimum ration cost as the objective function. Forages were given a cost of zero so that the amount of forage in the ration would be maximized. The INRAtion program, which also maximizes forage in the ration, was used to formulate rations using the INRA system. The NCPS is a ration evaluator, not a formulator. An iterative process was used to formulate rations using version 2.12D (06JUL95) software from the University of Pennsylvania with the feed library that was provided. First, each forage was included in the ration as the sole ingredient; then corn and soybean meal were substituted for forage until energy and metabolizable protein requirements were met and intake matched NCPS recommended amounts. Finally, minimal amounts of supplements were substituted for corn or soybean meal to meet the ammonia and peptide requirements of ruminal microorganisms.

Discussion

The NRC system predicted the lowest intake and, although net energy of lactation (NE_L) requirements were lower than other systems, the average energy density of the ration recommended by this system was high and

forage proportion was low (Table 1). Both the NRC and NCPS systems had high metabolizable protein requirements and required higher proportions of protein supplements (soybean and fish meals) in the average ration. The NCPS system yielded rations with the highest content of forages. It also obtained rations that were particularly high in grass forages and low in corn. The INRA and NDF/AP systems estimated similar high intakes of total feed with less protein supplements than required by NRC or NCPS. The NDF/AP system recommended lower levels of grass forage than INRA and NCPS and, because it predicted greater microbial metabolizable production, it yielded rations containing some urea (for the corn silage ration) and less protein supplements. Similar results were obtained for rations recommended for cows with lower production.

Conclusions

Because differences in animals and feeds are small, the discrepancies among systems indicate that there is a need to identify the strengths and weaknesses of each and develop a more accurate system to formulate rations for dairy cows that uses the best aspects of each system.

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Table 1. Average kilograms of ingredients in daily rations formulated for a high producing cow using different systems of animal requirements and feed nutritive values.

Ingredient/Nutrient	NRC	NCPS	NDF/AP	INRA
Alfalfa silage	2.52	3.67	3.66	3.99
Corn silage	3.37	4.50	4.04	3.80
Grass silage	2.48	4.50	2.78	3.83
Grass hay	1.98	4.26	2.60	4.03
Corn, ground	9.69	4.03	10.54	7.48
Soybean meal (44% CP)	2.29	2.45	0.56	1.41
Fish meal	0.27	0.27	0.00	0.19
Urea	0.03	0.04	0.19	0.04
Animal fat	0.08	0.00	0.00	0.00
Cottonseed hulls	0.15	0.00	0.00	0.00
Minerals	0.40	0.48	0.44	0.13
Total dry matter	23.27	24.20	24.89	24.89
Forage (% of ration dry matter)	45	70	53	63
NE_L (Mcal)	39.72	41.58	41.29	41.44
Bacterial metabolizable protein	1.655	1.610	1.736	1.217
Dietary metabolizable protein	1.155	1.244	0.942	1.231
Total metabolizable protein	2.810	2.855	2.678	2.448

Alfalfa Silage Versus Red Clover Silage or a Mixture of Alfalfa and Red Clover Silage as the Sole Forage for Lactating Dairy Cows

G.A. Broderick and S. Maignan

Introduction

The large proportion of CP in alfalfa silage (AS) that is present as NPN substantially reduces the efficiency of protein utilization by lactating dairy cows (Nagel and Broderick, 1992). Red clover silage (RCS) typically has a much lower content of NPN than AS (Albrecht and Muck, 1991). In a previous feeding study (Broderick and Sterrenburg, 1996 USDFRC Res. Sum.), we found that DMI and milk yield were greater on AS than on RCS, but this result was confounded by the fact that the CP content of the AS was seven percentage units greater than that of the RCS. Therefore, we repeated the comparison of AS versus RCS as the only forage for lactating cows, but with added solvent soybean meal (SBM) to equalize dietary CP content. Also, a third diet containing silage harvested from a co-culture of alfalfa and red clover (AS + RCS) was included in this trial. No supplemental bypass protein was used in this study. Our objective was to compare the lactational performance of cows fed AS, RCS or AS + RCS as their sole source of dietary forage.

Materials and Methods

A replicated 3 x 3 Latin square lactation trial was conducted. First cutting red clover was wilted to about 40% DM, chopped and ensiled in a concrete stave silo. Second cutting alfalfa was wilted to about 50% DM, chopped and ensiled in an upright tower silo. A mixture of alfalfa and red clover planted together, also from first cutting, was wilted to about 50% DM, then chopped and ensiled in a concrete stave silo. Based on the CP contents of initial samples of each silage obtained when the silos were opened, three diets were formulated with equal NE_L containing 60% DM from one of the forages, 32 or 36% DM from (unground) high moisture corn, plus sufficient SBM to give about 16.5% CP (Table 1). Twenty-one multiparous cows (including three with ruminal cannulae), averaging 65 DIM, were blocked into seven groups of three cows by DIM and randomly assigned to diets in replicated 3 x 3 Latin squares. Diets were fed for 4-wk periods before switching to the next diet (total 12 wk); production and intake data were analyzed from the third and fourth wk of each period. Apparent digestibility of DM and NDF was estimated from fecal grab samples using indigestible ADF as an internal marker. Ruminal sampling was done on the last day of each period.

Results and Discussion

The CP contents of the silages changed through the course of the trial — the AS declined and the RCS increased from the CP levels used to formulate the diets. Thus, the AS diet is slightly lower, and the RCS diet slightly higher, than the target of 16.5% CP; the AS + RCS diet contained very nearly the desired level of CP (Table 1). The relative CP levels of the AS and the RCS were similar to what was observed previously, when we found that RCS was about 1 to 2 percentage units lower in CP than AS. As expected, RCS had lower NPN: RCS was 19% and AS + RCS was 23% lower in NPN than the AS. The RCS and AS + RCS were, respectively, 2.3 and 4.2 percentage units lower in NDF than the AS (Table 1). Based on its chemical composition, AS + RCS was more like RCS than AS. Intake of DM was lower on the RCS and AS + RCS diets than on the AS diet, but apparent digestibilities of DM and NDF (estimated using indigestible ADF as internal marker) were higher (Table 2). Using these data, intakes of digestible DM were computed to be 14.4, 14.8 and 16.4 kg/d on the diets containing, respectively, AS, RCS and AS + RCS. There were no differences in milk composition or in production of milk and milk components among the three diets (Table 2). However, the similar milk yields at lower DMI resulted in significantly higher efficiencies (milk : DMI) on the RCS and AS + RCS diets.

Although milk urea and ruminal ammonia were not different among the three diets, concentrations of blood urea tended to be lower on the RCS and AS + RCS diets (Table 2), despite their higher levels of CP. This suggested that efficiency of utilization of CP was greater on the two diets containing RCS.

Summary and Conclusion

Results from this trial indicated that, although DMI was lower on diets containing RCS or AS + RCS as the sole forage, DM and fiber digestibility were greater and yield of milk and milk components was similar in cows producing about 33 kg of milk/d. Milk production per unit DMI was higher, and blood urea was depressed, suggesting that energy and CP utilization were more efficient on diets containing RCS or AS + RCS than on diets containing AS. These results suggest that the energy and protein in RCS may be used more efficiently than in AS.

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Nagel, S. A. and G. A. Broderick. 1992. Effect of formic acid or formaldehyde treatment of alfalfa silage on nutrient utilization by dairy cows. J. Dairy Sci. 75:140-154.

Table 1. Composition of forages and diets[1].

	Forage		
Item	AS	RCS	AS + RCS
DM, %	52.0	35.8	49.0
CP, % of DM	19.1	17.9	17.4
NPN, % of total N	50.0	40.3	38.7
NDF, % of DM	46.2	43.9	42.0
ADF, % of DM	36.2	33.6	31.9

	Diet		
Item	AS	RCS	AS + RCS
	- - - - - - - - - % of DM - - - - - - - - -		
Alfalfa silage	60.0	. . .	. . .
Red clover silage	. . .	60.0	. . .
Alfalfa + red clover silage	. . .	. . .	60.0
HMC	36.2	32.3	32.3
Soybean meal	2.7	6.6	6.6
Minerals & vitamins	1.1	1.1	1.1
Chemical composition			
CP	16.0	16.8	16.4
NDF	33	32	31
NE_L , Mcal/kg DM	1.59	1.59	1.59

[1]AS = alfalfa silage, RCS = red clover silage, AS + RCS = alfalfa plus red clover silage (grown together), HMC = high moisture corn (unground).

Table 2. Effect of feeding forage as alfalfa silage (AS), red clover silage (RCS) or a mixture of AS and RCS (grown together) on DMI, BW gain, apparent DM and NDF digestibility, production of milk and milk components, and concentrations of blood glucose, blood and milk urea, and ruminal pH and ammonia.

Item	AS	RCS	AS + RCS	SEM[1]	P > F[2]
DMI, kg/d	25.5[a]	23.0[b]	24.2[b]	0.4	0.011
BW change, kg/d	0.38	0.03	0.29	0.15	0.294
DM digestibility, %	56.3[c]	64.1[b]	67.6[a]	0.9	< 0.001
NDF digestibility, %	42.7[b]	49.9[a]	51.3[a]	0.5	< 0.001
Milk yield, kg/d	32.0	32.7	33.6	0.6	0.742
Fat, %	3.36	3.42	3.59	0.13	0.648
Fat, kg/d	1.08	1.12	1.21	0.04	0.817
Protein, %	3.04	3.02	3.03	0.02	0.284
Protein, kg/d	0.98	0.99	1.02	0.02	0.729
Lactose, %	4.77	4.81	4.81	0.03	0.859
Lactose, kg/d	1.54	1.58	1.63	0.03	0.820
SNF, %	8.52	8.55	8.56	0.03	0.514
SNF, kg/d	2.75	2.81	2.90	0.06	0.753
Efficiency[3]	1.27[b]	1.43[a]	1.40[a]	0.04	0.049
Blood glucose, mg/dL	55.0	55.9	54.5	0.5	0.086
Blood urea, mg N/dL	13.13[a]	12.89[ab]	12.40[a]	0.39	0.052
Milk urea, mg N/dL	9.92	10.46	9.60	0.40	0.292
Ruminal pH	6.15	6.05	6.15	0.05	0.232
Ruminal ammonia, mM	9.30	8.56	6.94	0.95	0.218

[a,b,c]Means within the same row without a common superscript differ ($P < 0.05$).
[1]SEM = Standard error of the mean.
[2]Probability of a significant effect of diet.
[3]Milk yield : DMI.

A Statistical Evaluation of Animal and Nutritional Factors Influencing Concentrations of Milk Urea Nitrogen

G.A. Broderick and M.K. Clayton

Introduction

Urea is the primary form in which N is excreted in mammals, and elevated concentrations of blood urea N (BUN) are known to reflect inefficient utilization of dietary CP. Urea equilibrates rapidly throughout body fluids, including milk, and concentrations of milk urea N (MUN) are closely related to BUN (Gustafsson and Palmquist, 1993). Therefore, MUN can serve as an easily sampled indicator of BUN. Last year, we reported on relationships, obtained by analyzing mean data from 22 feeding trials, of a number of dietary and milk yield factors to MUN concentration. Since that report, we have performed a more complete analysis using observations for individual cows rather than mean data. Also, data were added from 28 more diets fed in 13 additional trials. Our objective was to conduct a statistical evaluation on this data set to quantify: 1) the effect of various animal and dietary factors on the relationship between MUN and BUN, and 2) the value of MUN for assessing protein status of the lactating cow.

Materials and Methods

Data were collected in 35 conventional lactation trials conducted with 482 Holstein cows of known parity, BW and DIM and fed 106 different diets; intakes of DM, CP and estimated NE_L, change of BW, BUN concentrations, and production of milk, fat, protein and lactose was determined. A total of 2231 measurements of MUN and BUN was made during these studies. In 20 trials, ruminal NH_3 was measured in 50 cannulated cows fed 69 diets. In one trial, total urinary urea N concentration and excretion were determined and compared to MUN in AM and PM milk. Concentrations of MUN and BUN were determined in these trials using a diacetyl monoxime colorimetric assay adapted to the Technicon AutoAnalyzer. Linear and mixed effects regression models in SAS were used to study the relationship of: 1) BUN to plasma urea N (PUN); 2) MUN to BUN; and 3) MUN to various quantities. The number of samples necessary to determine mean MUN concentration within 95% confidence intervals of 1.0 and 2.0 mg N/dL also was estimated. Data from 27 trials already are published in nine papers, two manuscripts in press, one abstract, and one thesis; data from eight trials are unpublished.

Results and Discussion

Regression of data from two trials yielded a strong relationship of BUN to PUN ($r^2 = 0.952$) with slope not different from 1.0 and intercept not different from 0. Thus, BUN and PUN are virtually the same, and the term BUN can be used to describe urea concentration in both total blood and deproteinized blood plasma. The overall, mixed effects model for regression of MUN on BUN using all the data (Fig.1) indicated a strong correlation ($R^2 = 0.842$). Although the magnitude of slopes (0.62 versus 0.60) and intercepts (4.8 versus 5.1) was similar for the mixed effects model and a simple linear regression model, linear regression of MUN on BUN was not as well correlated ($R^2 = 0.588$). This is because the mixed effects model accounted for a significant cow-by-BUN interaction, whereby each cow had its own slope for MUN on BUN. It was expected that MUN and BUN would be highly correlated (Rook and Thomas, 1985). In our trials, only a single blood sample was taken from each cow 4 h after feeding. Gustafsson and Palmquist (1993) observed that urea in blood serum peaked about 3 h after feeding; therefore, BUN concentrations likely were near maximum at blood sampling time in our trials. This may explain the slope of 0.62 from our regression of MUN on BUN (Fig.1).

Single factors that yielded significant regressions on MUN concentrations using the mixed effects models were: dietary CP concentration [expressed either per unit DM (R^2 = 0.839) or per unit NE_L (R^2 = 0.833)], excess N intake (R^2 = 0.772), N-efficiency (R^2 = 0.626), and ruminal NH_3 (R^2 = 0.574). Urea in body fluids including milk probably reflects N inefficiency due to both excess protein degradation in the rumen and excess amino acid supply to the tissues. This may explain why MUN was better correlated to dietary CP content than to ruminal NH_3. When all factors were analyzed at once with the mixed effects model, 12 made significant ($P < 0.10$) contributions (Table 1): BUN, BW, FCM yield, dietary CP content, excess N intake, DMI, and DIM were positively related to MUN; parity, milk and fat yield, dietary CP/NE_L content, and NE_L intake were negatively related to MUN. Protein and SNF yield, dietary NDF and NE_L content, DM- and N-efficiency, and CP intake were not significant in the model. Thus, MUN concentrations will be influenced by multiple animal and dietary characteristics.

On the farm, milk often is sampled for DHI analysis at only one of the daily milkings. In two trials, we determined MUN in both AM and PM milk samples. Over both trials, BUN was associated more strongly to MUN in AM milk (R^2 = 0.686) than to MUN in PM milk (R^2 = 0.526); the two regressions had different slopes (P <0.02) and intercepts (P <0.0001). As expected, regression of mean MUN concentration on BUN explained more of the variation (R^2 = 0.737) in BUN than did MUN in either AM or PM milk. In one of these two trials, total urine collection and urinary urea N analyses were made for the 12-h periods corresponding to MUN in AM and PM milk (Table 2). Urine volume excreted during the 12-h preceding the AM milking was greater than that for the 12-h preceding the PM milking; the reverse was true for milk yield. Urinary urea N and MUN followed similar patterns in that concentrations of both were higher in PM than in AM secretions. As expected, urinary urea concentration greatly exceeded MUN: urea N was 38 and 32 times more concentrated in AM and PM urine than in AM and PM milk. Gonda and Lindberg (1994) found that urinary urea concentration averaged 39 times greater than MUN. Lower MUN concentrations in AM than PM milk resulted in lower amounts and proportions of total urea excretion in AM (1.8%) than in PM (3.3%) milk (Table 2). These data clearly indicated that MUN concentration patterns were not symmetrical over the two

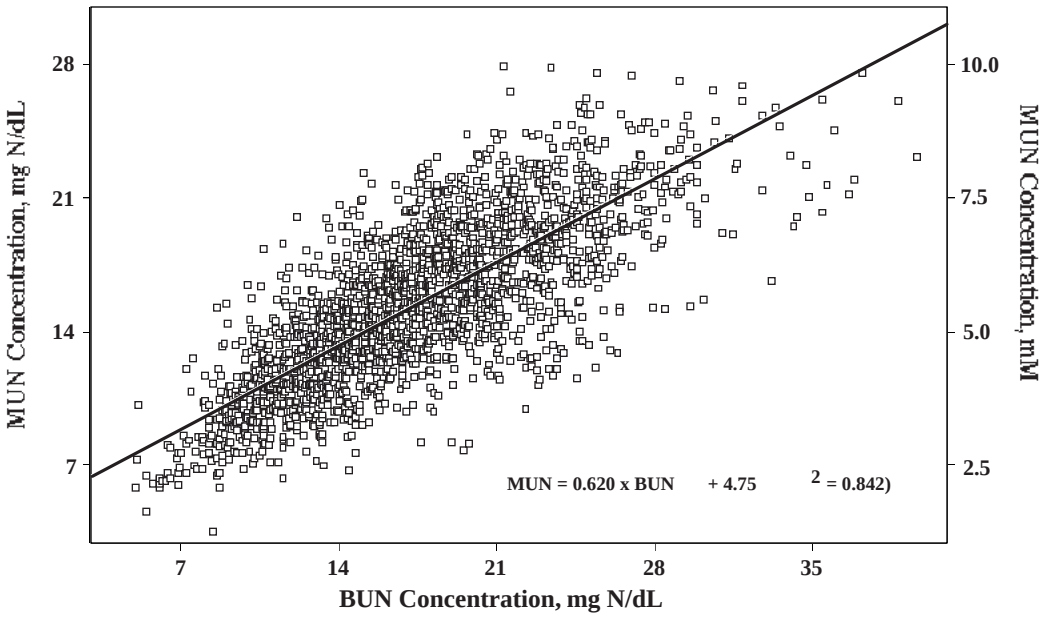

Figure 1. Regression of milk urea N (MUN) concentration on blood urea N (BUN) concentrations using all 2231 observations in the mixed effects model.

halves of the day and imply that switching milk sampling back and forth between AM to PM milkings is not appropriate. Composite milk samples representing the 24-h day will improve MUN reliability.

The numbers of cows fed a specific diet that must be sampled to determine the mean MUN concentration on that diet, within 95% confidence intervals of 1.0 or 2.0 mg N/dL, were estimated to be 16.5 and 4.1, respectively. This information may be used to develop recommendations for sampling milk for MUN analysis. Although the added precision gained by sampling 16 cows may not be necessary, our within-diet variation in MUN indicated that sampling milk from at least four cows would be the minimum needed to estimate MUN on a given diet. Milk samples representing the 24-h day will substantially improve reliability of MUN data. Switching sampling among AM to PM milkings, and presumably among more frequent 3X or 4X milkings, will confound interpretation of MUN data. Generally, sampling bulk tank milk probably would have little value unless used in conjunction with a dietary change that affected all the cows contributing milk to the tank. For example, if a lower protein alfalfa silage were replaced with one higher in protein such that dietary CP increased from 17 to 18% CP, the increase in MUN should be predictable. Rearranging the equation relating dietary CP% to MUN yields: MUN = (%CP - 13.7) / 0.269, increasing dietary CP by 1 percentage unit, will increase MUN by 3.7 mg N/dL in bulk tank milk.

Summary

Statistical analyses using both linear regression and mixed effects models were conducted on a large set of MUN data obtained in feeding studies with lactating dairy cows. Concentrations of BUN and MUN were found to be highly correlated. Level of MUN was more closely related to dietary CP concentration, expressed either on a DM or energy basis, than to N efficiency or ruminal NH_3. When all factors were analyzed at once with a mixed effects model, BUN, BW, FCM yield, dietary CP content, excess N intake, DMI, and DIM were positively related to MUN in the model; parity, milk and fat yield, dietary CP/NE_L content, and NE_L intake were negatively related to MUN in the model; protein and SNF yield, dietary NDF and NE_L content, DM and N efficiency, and CP intake were not significant in the model. In two trials, different relationships were found between BUN and MUN when assessed from MUN in either AM or PM milk collected in our twice daily milking scheme; BUN was more highly correlated to mean daily MUN concentration. Daily composite milk samples from 4 cows should be analyzed to estimate mean MUN concentration within a 95% confidence interval of +/- 2 mg N/dL.

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Table 1. Parameters making significant contributions to the regression of milk urea N (MUN) on using the multiple factor, mixed effects model.[1] Denominator df = 1249; R^2 = 0.875.[4]

Parameter or factor[2]	Estimated coefficient	SE	*t*	*P*[3]
MUN (mg N/dL) =				
Intercept	- 4.713	1.897	- 2.48	0.013
BUN (mg N/dL)	0.484	0.013	37.05	< 0.001
Parity	- 0.175	0.045	- 3.90	< 0.001
Body weight (kg)	0.003	0.001	2.55	0.011
Milk yield (kg/d)	- 0.101	0.028	- 3.63	< 0.001
3.5% FCM yield (kg/d)	0.187	0.053	3.52	< 0.001
Fat yield (kg/d)	- 1.802	0.940	- 1.92	0.056
CP (% of DM)	0.843	0.089	9.51	< 0.001
CP/NE_L (g/Mcal)	- 0.059	0.019	- 3.18	< 0.001
Excess N intake (g N/d)	0.007	0.003	2.59	0.010
DMI (kg/d)	0.103	0.055	1.88	0.061
NE_L intake (kg/d)	- 0.133	0.053	- 2.48	0.013
DIM	0.003	0.001	1.93	0.054

[1]BUN = Blood urea N; CP/NE_L = dietary CP/NE_L, where NE_L was computed from NRC (1989) tables; NE_L = NE_L intake computed from NRC (1989) tables; excess N intake = total N intake - milk N secretion.
[2]There were 2226 observations for each factor used in this model. Ruminal NH_3 was omitted from this model because of too few observations.
[3]Student's *t* and its associated *P*-value.
[4]Coefficient of determination determined for the mixed effects model.

Table 2. Concentration and excretion of urea N in urine and milk over 12-h periods ending at 4:00 AM and 4:00 PM.[1]

	12-h Period ending at		
Item	4:00 AM	4:00 PM	SEM[2]
Urine volume, L/12 h	20.4	14.7	0.4
UUN, mg N/dL	460.1	510.5	15.0
Urinary urea, g N/12 h	92.5	73.4	2.6
Milk volume, L/12 h	13.5	15.1	0.3
MUN, mg N/dL	12.0	16.0	0.37
Milk urea, g N/12 h	1.60	2.41	0.07
Total urea, g N/12 h	94.1	75.8	2.6
Milk urea/total urea, %	1.78	3.29	0.17

[1]UUN = Urinary urea N; MUN = milk urea N; SEM = standard error of the mean.
[2]Each AM versus PM comparison was significantly different ($P < 0.001$).

Effect of Replacing Alfalfa Silage with Red Clover Silage in the Diets of Lactating Dairy Cows

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Introduction

Alfalfa silage (AS) is the forage most commonly fed to dairy cows in the Midwest U.S. However, during ensiling as much as 60% of its CP can be converted to NPN. This formation of NPN in the silo substantially reduces efficiency of utilization of the CP in alfalfa silage (Nagel and Broderick, 1992). Red clover silage has been found to contain significantly less NPN (Albrecht and Muck, 1991). An enzyme system in red clover, polyphenol oxidase, converts several phenols that are also present in red clover into quinones (Jones et al., 1995); these quinones react rapidly with proteases, the enzymes that break down proteins in the silo. Thus, red clover silage (RCS) contains substantially less NPN than AS. The objective of this trial was to compare the protein value of AS and RCS when fed as the sole source of dietary forage to lactating dairy cows. Yields of milk and milk components were used as the indicator of the dietary protein status. Also, the forage serving as the better protein source should give rise to a smaller response in yield of milk and milk components with supplementation of bypass protein (from fish meal).

Materials and Methods

A replicated 4 x 4 Latin square lactation trial was conducted to compare the production of lactating cows fed either AS or RCS. Red clover silage was wilted to about 40% DM from two cuttings taken on 6/16/94 (first cutting) and 7/29/94 (second cutting) and ensiled in a concrete stave silo. Alfalfa silage was harvested from two cuttings taken on 8/30/94 (fourth cutting) and on 9/1/94 (third cutting) and ensiled in another concrete stave silo. Samples of both forages were collected as fed during the trial and analyzed for composition (Table 1). Four diets were fed (Table 1): two that contained 60% DM from either AS or RCS plus 36% DM from ground (3/8″ screen) high moisture corn (HMC), and two that contained the same basic ingredients except 3% ruminant grade fish meal (Sea-Lac) was substituted for some of the HMC. The only other difference in these diets was the source of forage; no attempt was made to equalize CP of the diets fed in this trial. Twenty-four cows (16 multiparous and 8 primiparous cows) averaging 59 DIM were blocked into six groups of four cows each by parity and DIM and randomly assigned to diets in balanced 4 x 4 Latin squares. Diets were fed for 3-wk periods (total 12 wk) before switching; production and intake data were analyzed from the second and third wk of each period. Four additional cows that were later in lactation and equipped with permanent ruminal cannulae were also used in a single 4 x 4 Latin square for ruminal sampling on the last day of each period.

Results and Discussion

The CP content of the two AS fed in this trial averaged 7.1 percentage units more CP than the two RCS; as a result, the RCS diets contained 4.2 percentage units less CP (Table 1). The two forages contained similar levels of NDF and ADF. In two previous trials, RCS was only 1.5 percentage units lower in CP than AS judged to be of "equal" maturity (i.e., about equal NDF content). As expected, RCS had substantially less NPN—only about 60% of that of AS (Table 1). Intake of DM was 1.5 kg/d lower on the two RCS diets and was not influenced by fish meal feeding (Table 2). Without fish meal supplementation, milk production was 1.5 kg/d greater on AS than on RCS, but there were no differences between these two diets in production of fat, protein, lactose and SNF (Table 2). Fish meal feeding resulted in similar increases in yield of milk, protein, lactose and SNF on both the AS and RCS diets; protein yield was increased on both diets by 70 g/day (Table

2). Previously, fish meal supplementation of AS was observed to increase protein yield by 100 g/d (Broderick, 1995). Interestingly, efficiency (milk : DMI) was greater on RCS than on AS; adding fish meal to the diet removed this difference in efficiency between RCS and AS. This suggests that, although diets were formulated to be equal in NEL, the availability of energy in RCS was greater than that in AS. Reduced blood and milk urea and ruminal ammonia on RCS were confounded by the much lower CP on the RCS diets (Table 2). A surprising finding was the elevated blood glucose on RCS which may be related to the significantly lower levels of milk fat on that forage (Table 2).

Summary and Conclusion

Results from this trial indicated that both DMI and milk yield were lower on RCS than on AS, but there were no differences in yield of milk components on these two forages. The magnitude of the response in milk and component yields to supplemental bypass protein (from fish meal) were similar for both AS and RCS, suggesting that protein status of the cows was similarly limiting on both forages. Greater production efficiency and slightly elevated blood glucose suggest availability of energy in RCS may be greater than that in AS. Dietary protein levels should be more nearly equal in future experiments designed to compare the nutritional value of these two forages.

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C = high moisture corn.

Table 1. Composition of forages and diets[1].

	Forage	
Item	AS	RCS
DM, %	36.1	42.0
CP, % of DM	23.1	16.0
NPN, % of total N	49.2	30.6
NDF, % of DM	43.3	44.7
ADF, % of DM	33.2	31.0

	Diets			
Item	AS	RCS	AS + FM	RCS + FM
	% of DM			
Alfalfa silage	60.3	. . .	60.3	. . .
Red clover silage	. . .	59.9	. . .	59.9
Ground HMC	36.2	36.6	33.2	33.6
Soybean meal	2.4	2.5	2.4	2.5
Fish meal	. . .	. . .	3.0	3.0
Minerals & vitamins	1.1	1.1	1.1	1.1
Chemical composition				
CP	18.4	14.1	20.2	16.0
NDF	32	32	32	32
NE_L , Mcal/kg DM	1.59	1.57	1.58	1.57

[1]AS = alfalfa silage, RCS = red clover silage, FM = fish meal, HMC = high moisture corn.

Table 2. Effect of feeding forage as alfalfa silage (AS) or red clover silage (RCS), with or without supplemental fish meal (FM) on DMI, BW gain, production of milk and milk components, and concentrations of blood glucose, blood and milk urea, and ruminal pH and ammonia.

Item	AS	RCS	AS + FM	RCS + FM	SEM[1]	P > F[2]
DMI, kg/d	24.2[a]	21.6[b]	24.1[ab]	22.4[b]	0.6	< 0.001
BW change, kg/d	0.16	0.48	0.34	0.48	0.12	0.206
Milk yield, kg/d	33.4[b]	31.9[c]	34.9[a]	33.7[b]	0.3	< 0.001
Fat, %	3.78[a]	3.44[b]	3.51[ab]	3.36[b]	0.11	0.047
Fat, kg/d	1.20	1.12	1.20	1.14	0.03	0.185
Protein, %	2.96[bc]	2.92[c]	3.02[a]	2.98[ab]	0.01	< 0.001
Protein, kg/d	0.95[b]	0.92[b]	1.02[a]	0.99[a]	0.01	< 0.001
Lactose, %	4.81[a]	4.84[a]	4.77[b]	4.81[a]	0.01	0.001
Lactose, kg/d	1.55[b]	1.53[b]	1.63[a]	1.62[a]	0.02	0.015
SNF, %	8.31	8.47	8.50	8.51	0.08	0.320
SNF, kg/d	2.74[bc]	2.68[c]	2.89[a]	2.84[ab]	0.04	0.002
Efficiency[3]	1.38[b]	1.47[a]	1.45[a]	1.51[a]	0.02	< 0.001
Blood glucose, mg/dL	52.9[bc]	54.1[ab]	52.2[c]	54.2[a]	0.4	0.003
Blood urea, mg N/dL	17.8[b]	7.9[c]	20.9[a]	10.7[c]	0.3	< 0.001
Milk urea, mg N/dL	17.7[b]	7.2[d]	21.3[a]	10.7[c]	0.3	< 0.001
Ruminal pH	6.02	6.06	6.15	6.06	0.04	0.289
Ruminal ammonia, mM	16.1[a]	4.2[b]	17.4[a]	4.9[b]	0.6	< 0.001

[a,b,c]Means within the same row without a common superscript differ ($P < .05$).
[1]SE = Standard error of the mean.
[2]Probability of a significant effect of diet.
[3]Milk yield : DMI.

Production Response to Feed Supplementation of Dairy Cows in a Seasonal Calving and Grazing System

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Introduction

Providing supplemental feed under grazing conditions usually supports higher milk production. The objective of this study was to measure the impact of feed supplementation on milk yield, milk composition and reproductive performance of Holstein dairy cows in a spring calving and grazing system.

Materials and Methods

A two-year grazing study was conducted with Holstein dairy cows at the U.S. Dairy Forage Research Center, Prairie du Sac, WI. In 1994 and 1995, 50 to 54 cows were randomly assigned before calving to one of three treatment groups. The three treatment groups were all pasture (P), 2/3 pasture (2/3P), and 1/3 pasture (1/3P). Cows calved between March and June each year. Cows in P, 2/3P and 1/3P groups were fed diets containing forage and grain in a ratio of about 100:0, 75:25 and 50:50 (% DM basis) until pasture was ready. In 1994, alfalfa silage was the sole forage source during the pre-grazing period, whereas in 1995 alfalfa silage and corn silage were used in a 2:1 ratio. During the grazing season, cows were slowly changed (over a period of 5 days) from the indoor diets to pasture. Most of the cows (90%) were on pasture by May 16. During the grazing season, cows in P, 2/3P and 1/3P groups consumed all, 2/3 and 1/3 of their daily feed from the pasture, respectively. The balance of feed for the 2/3P and 1/3P groups was supplied by a supplement.

Ingredient composition of the supplements is in Table 1. Cows grazed permanent pasture containing primarily a mixture of *Poa pratensis* L. (Kentucky bluegrass), *Elytrigia repens* (L.) Nevski (quackgrass), *Bromus inermis* Leyss (smooth bromegrass), and *Trifolium repens* L. (white clover). Cows in the three treatment groups were grazed as a single group. Pasture was managed under an intensive rotational grazing system. Cows were offered a new paddock daily after the morning milking. The amounts of supplement fed to the 2/3P and 1/3P groups during 1994 were adjusted weekly according to milk yield of the previous week and averaged 5.6 and 10.5 kg/cow/d, respectively. In 1995, cows were fed a constant amount of supplement (6.0 and 11.6 kg/cow/d for the 2/3P and 1/3P groups, respectively) throughout the grazing season. Supplement was fed after each milking. Cows grazed from May 16 to October 21, 1994 and from May 16 to September 30, 1995. Total grazing area varied between 30 to 50 acres over the grazing season. During each grazing cycle, forage samples were collected from three paddocks before grazing to monitor quality. Forage samples were dried and analyzed for crude protein (CP) and fiber. After the grazing season was over, cows were housed in a freestall barn and were fed diets containing forage to grain in ratios similar to those used before grazing. Cows were synchronized for breeding using GnRH and PGF2a. Cows were dried off on January 30, 1994 and on January 15, 1995. Some cows were dried off before these dates to give 53 days before the expected calving date. Daily milk yield was recorded. Milk samples were analyzed weekly for composition.

Results and Discussion

Botanical composition of the pasture clipped to within 3 cm above the ground was (DM basis): grasses, 55%; white clover, 12%; weeds, 8%; and dead matter, 25%. Forage from the pasture contained an average CP, 14.7% ± 3.7 SD; NDF, 58.7% ± 4.0 SD and ADF, 36.8% ± 4.7 SD in 1994, and CP, 19.0% ± 3.9 SD; NDF, 50.6% ± 7.5 SD and ADF, 25.8% ± 4.6 SD in 1995. The number of cows used in each treatment and

experimental results are summarized in Table 2. Average days in milk were 275. Total milk yield increased with increasing amounts of supplement. Cows in P, 2/3P and 1/3P treatment groups produced an average 17.3, 21.2 and 26.3 kg of milk/d, respectively. Milk fat content was reduced in the 1/3P treatment during 1994. This decrease probably was due to the presence of corn silage and fine ground corn in the supplement. During 1995, alfalfa hay was used instead of corn silage, and milk fat content was not different among treatments. Milk protein contents were similar among treatments. Cows in the P group had lower body condition score and more reproductive failure than cows fed supplement.

Conclusion

Providing supplemental feed in a seasonal calving and intensive rotational grazing system increased milk yield and improved the reproductive performance of dairy cows. The highest level of supplementation in this experiment would be justified with North American feed and milk prices.

Table 1. Ingredient and chemical composition of the supplements (% DM basis).

	Treatment groups			
	2/3P	1/3P	2/3P	1/3P
Ingredient	1994		1995	
Alfalfa hay	-	-	50.0	25.0
Corn silage	33.3	25.0	-	-
High moisture ear corn	30.1[1]	42.0[1]	28.4[2]	48.3[2]
Soybean meal	-	3.0	-	6.0
Roasted soybean	24.0	18.0	18.0	18.0
Linted cottonseed	8.0	9.0	-	-
Sodium bicarbonate	-	.45	-	-
Minerals and vitamins	4.6	2.5	3.6	2.7
Crude protein, % of DM	15.7	15.2	19.5	19.6

[1]Fine ground
[2]Coarse ground

Table 2. Influence of grain supplementation on lactation performance of dairy cows in a seasonal calving/grazing system.

	Treatment groups[1]					
	P	2/3P	1/3P	P	2/3P	1/3P
Item	1994			1995		
Number of cows	17	18	15	17	17	20
Supplement intake, kg/d	-	5.6	10.5	-	6.0	11.6
Average days in milk	265	272	265	286	282	281
Total milk yield, kg	4437[c]	5649[b]	6884[a]	5075[c]	6023[b]	7344[a]
Milk yield, kg/d	16.8[c]	20.8[b]	26.0[a]	17.7[c]	21.5[b]	26.5[a]
Milk fat, %	3.80[a]	3.63[a]	3.30[b]	3.60	3.50	3.40
Milk protein, %	3.00	3.02	3.06	3.01	2.92	3.01
Cows pregnant by 100d, %	41	56	53	18	41	45
Cows pregnant with first two services	8	11	9	5	7	12

Means in the same row with different superscripts within year differ ($P < .01$).
[1]Cows consumed all (P), 2/3 (2/3P) and 1/3 (1/3P) of their daily feed from pasture. Balance of feed for 2/3P and 1/3P groups was supplied by a supplement containing grain.

Energy Supplementation Sources for Lush Pasture

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Introduction

Lush, vegetative forage as found in well-managed pastures is generally high in protein content. The high protein concentration in lush pasture can pose a ration formulation problem because of the high degradability of most forage proteins. Excess ammonia production from forage protein degradation in the rumen can result in insufficient non-ammonia nitrogen flow to the small intestine to support genetic potential for milk production by dairy cows. One method to overcome this problem is to supplement grazing cows with ruminal undegradable protein sources. However, this solution results in waste of forage protein, additional cost for supplemental feed, and increased nitrogen load on the farm. A more preferable solution would be to supplement cows on lush pasture with energy sources that maximize microbial protein synthesis and capture of nitrogen from rumen degraded forage protein. This experiment examined three different supplemental energy sources for microbial protein synthesis in a continuous culture system.

Materials and Methods

Forage was collected from a rotationally grazed pasture and freeze-dried. The pasture consisted of a complex mixture (approximately 50:50) of legume and grass species. As shown in Table 1, the forage was high in protein and had moderate levels of fiber and energy. Based on preliminary in situ experiments, three energy supplements (corn grain, beet pulp, and soybean hulls) were selected because they were isocaloric but differed in type and rate of carbohydrate fermentation. Protein levels in the energy supplemented diets were similar, but lower than for the all forage diet. Continuous culture fermenters were fed either forage alone or forage plus energy supplement in a 55:45 forage to supplement ratio. Fermenters were fed forage every two hours. Energy supplements were fed two times per day, at 12-h intervals, to mimic a twice-a-day milking situation where supplemental feed is provided after milking. Fermenter pH was allowed to fluctuate, but NaOH was used to prevent the pH from dropping below 6.0. The fermenters were operated in a single-flow mode with flow rate maintained at 0.07/h. Effluent was collected for determination of feed digestibility, bacterial protein flow, and VFA and ammonia concentrations.

Results and Discussion

The three energy supplemented diets resulted in lower average fermenter pH levels than the all forage diets (Table 2), but even the all forage diets resulted in large amounts of volatile fatty acid (VFA) production that required addition of NaOH to maintain fermenter pH above 6.0. Corn supplementation required the largest amount of NaOH addition to maintain pH. The acetate to propionate (A:P) ratio of VFAs was greatest for the all forage diet and least for the corn supplementation, and the soybean hull diet resulted in similar A:P ratios as the all forage diet. As expected, addition of energy supplements to the forage resulted in reduced ammonia concentrations in the fermenters, with the corn and beet pulp diets causing the greatest reductions (Table 2). Because the forage basal diet supplied most of the protein in the diets, no differences were detected among diets in protein digestion. Neutral detergent fiber and cell-wall polysaccharide digestibilities were highest for the soybean hull diet and lowest on the corn grain diet. Non-structural carbohydrates digestibility was greatest for the corn diet. While efficiency of bacterial protein synthesis based on amount of organic matter digested was the same across all diets, the corn and soybean hull diets improved the utilization efficiency of degraded feed protein (Table 2).

Addition of energy supplements to lush pasture diets may reduce nitrogen losses from readily degradable forage proteins by allowing more

efficient capture of degraded protein as bacterial nitrogen. A readily fermentable fibrous energy supplement such as soybean hulls may be preferable to starch supplements such as corn because of less acid production and a higher A:P ratio of fermentation products.

Table 1. Chemical composition of dietary treatments.

	Diets			
Component[a]	Pasture Alone	Corn Grain	Beet Pulp	Soybean Hulls
CP	18.2	14.7	15.2	15.3
NDF	47.2	32.5	46.6	54.6
CWP	32.6	23.0	37.1	41.2
NFC	20.1	42.6	25.9	19.0
NSC	10.6	36.6	12.7	9.7
NE_L, Mcal/kg	1.54	1.68	1.65	1.65

[a]Components are in percent of DM unless indicated otherwise; CP (crude protein), NDF (neutral detergent fiber), CWP (cell-wall polysaccharides), NFC (non-fibrous carbohydrates), NSC (non-structural carbohydrates), and NE_L (net energy for lactation).

Table 2. Digestibility, fermentation characteristics, and efficiency of bacterial-nitrogen synthesis of dietary treatments.

	Diets			
Component[a]	Pasture Alone	Corn Grain	Beet Pulp	Soybean Hulls
Fermentation Characteristics				
pH	6.10^{b}	6.02^{c}	6.04^{c}	6.03^{c}
VFA, mM	126^{b}	142^{c}	124^{b}	152^{c}
A:P Ratio	4.01^{b}	2.46^{c}	3.05^{cd}	3.38^{bd}
NH_3, mg 100 ml^{-1}	10.3^{b}	2.1^{c}	2.6^{c}	4.4^{d}
Digestibility, %				
OM	37.8^{b}	54.8^{c}	45.8^{d}	46.2^{d}
CP	46.0	41.7	45.2	43.5
NDF	28.1^{b}	21.9^{c}	29.7^{b}	44.1^{d}
CWP	43.8^{b}	41.5^{c}	51.9^{d}	56.1^{e}
NFC	53.1^{b}	80.1^{c}	71.8^{c}	60.8^{bc}
NSC	64.9^{bc}	79.0^{b}	61.0^{c}	65.5^{bc}
Bacterial-N Synthesis Efficiency				
g N/kg OM digested	27.7^{b}	21.2^{c}	20.6^{c}	24.1^{c}
g N/kg N degraded	64.2^{b}	79.7^{c}	69.1^{d}	77.7^{c}

[a]VFA (volatile fatty acids), A:P (acetate:propionate), other abbreviations as defined in Table 1.

Effects of Acidified Fermentation By-Products and Anionic Salts on Acid-Base Status of Non-Lactating Dairy Cows

D.B. Vagnoni and G.R. Oetzel

Introduction

Milk fever (parturient paresis or parturient hypocalcemia) is an economically important disease of dairy cattle. Risk for developing milk fever is strongly influenced by pre-calving diet. Feeding acidogenic diets prior to calving has repeatedly been shown to significantly reduce the risk of milk fever. Diets can be made acidogenic either by addition of mineral acids or by supplementing with anionic salts. Such diets create a metabolic acidosis which is reflected in decreased urinary pH, increased urinary acid excretion, decreased blood bicarbonate concentrations, and decreased blood base excess. Changes in blood pH are minor because the acidosis is compensated. Acidosis improves calcium metabolism in parturient dairy cattle via bone buffering and improved vitamin D metabolism.

Dried condensed extracted glutamic acid fermentation product and dried corn fermentation solubles have been combined recently to create an acidified fermentation by-product feed (AFBP) commercially marketed as Bio-Chlor™. The AFBP is acidic because the glutamic acid and corn fermentation processes are stopped with strong acids (hydrochloric and sulfuric acids, respectively). No published studies in dairy cattle have evaluated the potential of an AFBP to influence acid-base balance and potentially reduce the risk of milk fever.

Materials and Methods

Eight pregnant, non-lactating, ruminally cannulated Holstein cows were assigned to two replicated 4 x 4 Latin squares with 14 day periods. Square 1 consisted of cows that had completed one lactation and square 2 consisted of cows that had completed either two or four lactations. Cows within squares were randomly assigned to treatment sequences. During week 1 of each period, cows received the control diet; and during week 2, they received one of four treatment diets - AFBP; $MgSO_4$ and NH_4Cl combination; $MgSO_4$, $CaCl_2$, and $CaSO_4$ combination; or the control diet. All diets except the control diet were approximately equal in acidogenic potential measured as dietary cation-anion difference (DCAD). These diets were all anionic, as evidenced by their negative DCAD values. Both the anionic diets and the control diet were formulated to provide similar amounts of energy (NE_L), crude protein, neutral detergent fiber, acid detergent fiber, Ca, P, and Mg. Diets were fed as a total mixed ration for ad libitum feed intake. Dry matter intake was measured daily.

Total urine collections were made using indwelling Foley catheters which were inserted on the next to last day of each experimental period. All urine produced during a 24 hour period was collected into 60 L plastic containers placed in ice baths. Urinary pH, volume, strong ion content, and net acid excretion (calculated from urinary bicarbonate, NH_4, and titratable acidity determinations) were measured. Blood samples for blood gas analysis (blood pH, pCO_2, bicarbonate, and base excess) were also collected on the last day of each experimental period.

Samples of whole ruminal contents were obtained at 0, 1, 3, 6, and 9 hours post-feeding on the last day of each experimental period. Ruminal pH, NH_4, and VFA concentrations were measured.

Results and Discussion

Dry matter intakes were very high for all diets (Table 1) compared to expected intakes for non-lactating Holstein cows. Intakes for the anionic diets were significantly lower compared to the control diet. All of the anionic diets tended to lower blood pH and pCO_2, but the differences were not significant. The anionic diets significantly decreased blood bicarbonate and

base excess values. Anionic diets also significantly increased urinary volume, reduced urinary pH, increased urinary net acid excretion, increased urinary Ca excretion, and decreased urinary strong ion difference. The AFBP treatment significantly reduced urinary magnesium excretion. Although significant differences between anionic diets were inconsistent, the numerical ranking of treatment means for measures of acid-base status consistently indicated that the magnitude of acidosis was greatest for AFBP; intermediate for $MgSO_4$ and NH_4Cl; and lowest for the $MgSO_4$, $CaCl_2$, and $CaSO_4$.

All of the anionic diets significantly lowered ruminal pH and increased ruminal NH_4 concentrations; however, the magnitude of the effects was small relative to the normal range for ruminal pH and NH_4. Increased NPN found in the anionic diets may explain the increase in ruminal NH_4. Individual concentrations of VFA were not affected by dietary treatment.

Conclusions

Acidified fermentation by-products were at least as effective as, if not slightly better than, conventional anionic salts in producing an acidic response in non-lactating dairy cattle. Therefore, these products have the potential to reduce the risk of milk fever if fed prior to calving. All anionic diets reduced dry matter intake, which may have been a response to the systemic acidosis they induced. Anionic diets exerted only modest effects on ruminal fermentation.

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Table 1. Effect of acidified fermentation by-products and anionic salts on whole blood, urine, and ruminal fluid parameters.

	Dietary treatment					Probability	
Item	Control	AFBP[1]	$MgSO_4$ + NH_4Cl	$MgSO_4$ + Ca salts	SEM	Diet	Control vs. anionic
Feed intake:							
DMI, kg/day	16.0[a]	14.4[c]	15.4[ab]	14.9[bc]	0.26	.004	.002
Whole blood:							
pH	7.42	7.41	7.41	7.39	0.01	.449	.232
pCO_2, mm Hg	40.7	37.7	39.1	40.7	1.7	.590	.460
HCO_3^-, mM	26.0	23.7	24.2	24.6	0.6	.105	.021
Base excess, mM	1.69	-0.56	-0.18	-0.04	0.57	.065	.010
Urinary output:							
Liters/day	14.6[b]	15.3[b]	17.1[a]	15.0[b]	0.4	.002	.020
pH	8.33[a]	6.37[c]	6.89[bc]	7.20[b]	0.20	<.001	<.001
NAE[2], meq/day	-2843[b]	185[a]	-28[a]	-202[a]	123	<.001	<.001
Ca, g/day	0.92[b]	6.70[a]	7.87[a]	6.87[a]	0.78	<.001	<.001
Mg, g/day	6.94[a]	4.51[b]	7.56[a]	7.03[a]	0.42	.001	.257
SID[3], meq/day	4243[a]	1181[b]	1777[b]	1129[b]	218	<.001	<.001
Ruminal fluid:							
pH	6.23[a]	6.07[b]	6.14[b]	6.12[b]	0.03	.008	.002
NH_4, mM	11.7[b]	14.0[a]	14.0[a]	13.6[a]	0.68	.103	.016
Total VFA, mM	131[a]	121[b]	124[b]	135[a]	2.1	.001	.106

[abc] Means with different superscripts within row differ ($P < .05$)

[1]AFBP = acidified fermentation by-products feed.

[2]NAE (net acid excretion) equals ammonium plus titratable acidity minus bicarbonate.

[3]SID (strong ion difference) equals (Na + K) - (Cl + S).

Farm/Herd Report - Wisconsin

U.S. Dairy Forage Research Center - Annual Field Operations Report January 1997

R.P. Walgenbach

As in 1995, the 1996 growing season began with very cold temperatures. But in contrast to the hot 1995 season, cool temperatures prevailed through most of the 1996 growing season. On 15 May only 5 growing degree days had accumulated and no corn had emerged. Air temperature seldom exceeded 80 degrees F. Rainfall in April was below normal. Rainfall in May was above normal and its pattern caused delayed corn and soybean planting. Rainfall recorded at the farm entrance rain gauge in inches was 1.64 in April, 4.1 in May, 7.83 in June, 3.44 in July, 1.97 in August, 0.88 in September and 3.29 in October. Between 16 and 18 June, 4.35 inches of rain fell at the farm. In Baraboo, just to the north of the farm, rainfall amounts of 7 to 10 inches were recorded. This rain flooded many fields in our area resulting in loss of crops. At the research farm we were fortunate to lose only about 10 acres of soybeans and 4 acres of corn. Soils in most fields sustained nitrogen (N) leaching and anaerobic N losses. The 1995/96 winter was extremely cold, and it also produced some January/February ice sheeting that killed or injured alfalfa in several areas of Minnesota and Wisconsin; however, alfalfa at the research farm tolerated the winter fairly well. The cold April and May temperatures slowed growth of alfalfa and delayed the start of first crop harvest into early June. We planted 66 acres of barley, 233 acres of soybeans, 369 acres of corn, 77 acres of spring and 66 acres of summer seeded alfalfa. The prior year's seedings of alfalfa totaled 293 acres. Barley planting started on 18 April and was completed on 23 April. All barley was no-till planted at 110 pounds per acre into soybean stubble with a John Deere 750 no-till drill. Prior to seeding, parts of some fields were rotary hoed to facilitate drying of soil surface insulated by soybean residue and manure. We spread about 8,000 gallons per acre of liquid manure on all barley fields. We no-till seeded alfalfa from 11 April to 7 May into fields that had corn harvested for silage in 1995. These fields also had 10,000 gallons of manure applied per acre. On 9 and 10 August we no-till seeded about 13 pounds per acre of alfalfa into harvested barley fields. We planted corn at about 33,500 to 43,000 seeds per acre from 23 April to 22 May. About 10 acres of grass sod were planted following conventional tillage, 85 acres of heavily manured corn residue were planted following one pass with a disk and 274 acres were planted no-till. We applied from 9,000 to 13,000 gallons of liquid manure per acre to most corn ground. All corn received 90 pounds per acre of 5-14-42 starter fertilizer and 160 pounds per acre of nitrogen from a combination of soybean, alfalfa and manure nitrogen (N) credits and surface applied 28% N. We no-till seeded soybeans in 7.5 inch rows at about 225,000 seeds per acre from 6 to 11 May.

Timely planting and nearly ideal growing temperatures produced barley yields that averaged 81.7 bushels per acre and ranged from 72.5 to 83.9 bushels per acre. We harvested barley from 23 to 27 July and stored it as a high moisture grain. Soybeans produced an average yield of 47.1 bushels per acre with a range of 32.7 to 63.2 bushels per acre. Soybean harvest occurred from 1 to 14 October. Excessive rain early in the growing season and very little rain during pod fill dramatically reduced soybean yields this season compared to those of 1994 and 1995 which were near 60 bushels per acre. Toward the end of the growing season, soybean plants on most knolls in all fields died prematurely due to lack of moisture. In addition to moisture stress, soybean plants in one particular field and in large areas of several fields exhibited severe symptoms of white mold.

Currently no soybean cultivars are resistant to white mold. This disease has many hosts and is affected very little by crop rotations.

About 2,779 wet tons of corn silage were harvested from 130 acres between 9 September and 10 October. Yields ranged from 6.7 to 8.3 tons of dry matter (DM) per acre and averaged 7.5 tons of DM per acre. We harvested about one third of our corn silage with a chopper equipped with a kernel processor that was loaned to the DFRC farm by John Deere to facilitate research with processed corn silage. We harvested approximately 165 acres of high moisture ground ear corn (HMGEC), 25 acres of HMG shelled (SC), and 49 acres of SC from 8 October to 8 November. The shelled corn (85% DM) equivalent yields averaged 166.5 bushels per acre and ranged from 132 to 183 bushels per acre. The total amount of HMGEC harvested was 1,488 tons adjusted to 29% moisture content. The total amount of HMGSC harvested was 151 tons adjusted to 29% moisture content. All non Bt corn was sprayed for corn borers this season. Corn on knolls in all fields exhibited symptoms of moisture stress and produced very small ears. But in general corn was able to obtain moisture in amounts sufficient to produce excellent yields this season. I also think that corn in this season benefited from availability of late season organic N provided by manure and legumes. The harvested alfalfa from established fields yielded an average of 4.1 tons DM per acre that ranged from 3.3 to 4.9 tons DM per acre. The cold spring delayed regrowth initiation and slowed subsequent growth which reduced first harvest yields. Most first crop yields were less than 1.8 tons DM per acre. Typically first crop yields ranged from 2 to 2.5 tons DM per acre.

Gene Dyar, Cluster Environmental Protection Specialist, continues to work on our fuel contamination located behind the milkhouse. Gene is optimistic that this site will be closed out with no need of major remediation. We hope to have final approvals early in 1997. I think that we have corrected the problems caused by lightening and electrical surges at both gate locations. Dan Mann of Wisconsin Power and Light discovered that the two gate houses containing our communication equipment were improperly grounded. Since these buildings were properly grounded, we have not had any malfunctions of modems.

Projects completed this past season include the blacktopping of the road around our expanded bunkers and the drive pad areas in front of these bunker silos. We also removed about three quarters of a mile of overgrown tree lines between fields. These tree lines will be replanted with tall prairie grasses to improve habitat for game birds, such as pheasants. This project will reduce the labor needed to limb trees and to remove fallen trees from fields. It also will greatly enhance the crops' use of sunlight and moisture along these areas. The Center purchased a John Deere 750 no-till grain drill, a Brent GT 440 gravity box with running gear and a used Ford truck with a grain/forage box. With these purchases, the research farm now owns all of the equipment used in field operations. Equipment purchased to replace existing equipment consisted of two Model E Huskey manure tanks, a White Model 6106 corn planter, a Model 6200 forage harvester and Model 6221 feeder housing for our Uni harvesting system, and a Model 773 Bobcat skidsteer loader.

In April, Dave Sprecher, the Ag. Project Supervisor at the research farm, resigned his position to pursue new challenges. Dave has contributed invaluable service to the facility operation and to many research projects conducted over the years. Dave approached projects with enthusiasm and energy that went beyond normal expectations. His ingenuity and creativity in solving problems and his organizational skills are assets that benefited the Research Center in countless ways. We thank Dave for his many contributions and wish him the best in his future endeavors.

This past season, corn and soybeans were planted past midnight and haylage was hauled and packed in the bunker at 10:30 P.M. more than once. It was that kind of year, and our employees responded to these challenges as usual. I thank all of them for their past and continued efforts and flexibility as we work to meet our objectives.

U.S. Dairy Forage Research Center - Annual Dairy Operations Report
January 1997

L.L. Strozinski - Herd Manager

Herd Statistics		Change from previous year
Herd Inventory		
Milking cows	300	0
Dry cows	50	+15
average cow age	45 months	0
percent first lactation	41%	- 5
percent second lactation	29%	+ 8
percent third lactation	15%	+ 1
percent greater than third	15%	- 2
Herd replacements	315	- 5
Total	**665**	+ 10
Rumen fistulated cows	32	
Herd Performance		
Cows calved	368	+ 2
Heifer calves born	160 live + 17dead	(+ 5)
Bull calves born	179 live + 18 dead	(- 5)
Heifer calves died < 1 year old	3 (1.87%)	
DHIA rolling herd average		
milk	20,337 lbs.	+ 429
protein	651 lbs.	+ 30
fat	747 lbs.	+ 19
Milk sold in 1996	6,298,921 lbs.	+ 84,909
Heifer calves sold	9	- 5
Bull calves sold	179	- 9
Cows sold	131	- 12
Cows culled for:		
reproduction problems	43	+ 1
poor production	14	- 7
poor udder	20	+ 1
poor feet and legs	9	- 9
mastitis	23	+ 11
injury	5	0
other	17	+ 4
Cattle sales revenue	$54,958.93	- 14,241.07
Herd Reproduction		
Average days open	123	+ 9
Average calving interval	13.02 months	+.27
Average services per conception	2.1	- .4
Average age at first calving	24 months	- 1

The 1996 USDFRC dairy herd activities can best be described as “business as usual.” Numbers of mature cows in the herd increased by 15 while the present number of herd replacements on hand is down by five. Overall animal numbers are up slightly from a year ago. Milk production has increased in 1996. Our DHIA rolling herd average for milk is at an all time high of 20,337 pounds. Current average production per cow per day is 73 pounds. The farm “mailbox” net price

received per hundred weight of milk ranged from $13.811 to $16.907 in 1996. Higher production coupled with favorable prices during most of the year resulted in increased milk revenues. Cattle prices, however, have remained very weak in 1996. It was not uncommon to net less than $5.00 for an 80 pound bull calf after associated sale and trucking costs at conventional cattle markets. Consequently, some bull calves were sold directly from the farm to area individuals to avoid sales costs and increase net cattle sales revenues.

Research activities with the herd continued at a high level in 1996 with 370 milking animals involved in 14 different trials. With increased cattle numbers and research activities, the dairy operation felt the effects of last year's reduction in staff. Although modifications and elimination of some work tasks have helped to streamline the work flow and allow us to meet the basic needs of the operation, there is a certain attention to details that has declined somewhat. This decline is haunting us in the form of poorer overall facility cleanliness, animal cleanliness and associated animal health issues. Time to give attention to all research trial details is also limited, and I feel that the overall support we are able to give to the research mission is less than it has been in the past. These developments have initiated action to add one full time employee back into the dairy operation.

Improvement and expansion projects for 1996 were also somewhat limited by the labor shortage. The new feed pad and manure handling system which were added at the hay storage shed in 1995 are working well for winter housing of 75 heifers and dry cows. Plans have been drawn and bids are in place to construct a free stall facility for 48 animals adjacent to the hay shed. These animals will be bedded with sand and will utilize the same feed pad area. The cattle mound has been relocated to make space for the new facility. The modification of the free stalls in the existing facility to improve cow comfort which was started in 1995 has not yet been completed due to lack of labor. This project remains on the plans for 1996.

Two changes are taking place in the milking operations. An order has been written to upgrade our milk meters, samplers and associated software. The new system will reduce maintenance cost, improve clean-in-place washing, improve sampling procedures, and increase accuracy. The other change in the system is a cooperative project with Dairy Equipment Company, the University of Wisconsin milking lab and Wisconsin Power and Light. This project involves the installation and evaluation of a variable speed vacuum pump system.

A significant management change made in 1996 was to divide and feed the dry cows in two separate groups. One group is for recently dry cows while the other is for cows due to calve in three to four weeks. This practice has significantly decreased incidence of milk fever and displaced abomasums in our herd.

The farm continues to be a popular place to visit and we continue to host many local, national and international guests each year. In 1996 an extra outreach effort was made to increase the visibility of the farm and make better use of the new conference room. Some of the activities hosted were: Professional Dairy Producers of Wisconsin Dairy Skills Workshop; Monsanto-Protiva Dairy Improvement Tour; Gromark Dairy Feed Salespeople Workshop; Sauk County Holstein Association Twilight Meeting.

The state of the dairy operation in 1996 has meant that many of the employees have had to put in extra time and effort not to mention alter their personal plans for time off to "get the job done." Many of these efforts are beyond the call of duty. I encourage the USDA-ARS, USDFRC and the University of Wisconsin to join me in recognition and appreciation of their efforts. I also extend my appreciation to the USDFRC field operation's crew for their assistance with chores and special projects throughout the year.

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